

# Rabies Virus in Raccoons, Ohio, 2004

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In 2004, the raccoon rabies virus variant emerged in Ohio beyond an area where oral rabies vaccine had been distributed to prevent westward spread of this variant. Our genetic investigation indicates that this outbreak may have begun several years before 2004 and may have originated within the vaccination zone.

Several wild carnivorous mammals may be competent zoonotic reservoirs for rabies viruses (1). Similar to how parenteral vaccination has contributed to control and elimination of rabies in dogs, effective oral rabies vaccines and application methods for wildlife species, most notably the red fox (*Vulpes vulpes*), have led to regional containment and elimination of the rabies virus variants associated with this species in large parts of Canada and Europe (2). The first step toward reducing the size of areas in which rabies is enzootically transmitted is containment of its regional spread. Understanding the conditions under which containment of wildlife rabies can reliably be achieved will facilitate the long-term goal of eliminating particular rabies virus variants from their respective reservoir species.

During the late 1970s, the range of a raccoon (*Procyon lotor*)-specific rabies virus variant (RRV) expanded substantially from the historically affected southeastern United States to the currently affected eastern North America (3). In 1996, to contain westward expansion of this variant, oral rabies vaccine (ORV) was distributed in Ohio. The ORV strategy includes distributing bait containing a vaccinia-rabies glycoprotein recombinant vaccine (4) while taking advantage of physiogeographic impediments to rabies transmission, such as mountains, rivers, and major highways to create a barrier 50 km–150 km wide between unaffected and enzootic areas.

During 1999–2004, ORV had apparently limited further spread of the virus (5) (Figure 1). However, in July 2004, RRV was diagnosed in a raccoon northwest of the ORV zone in Lake County, Ohio. As of December 2005, enhanced surveillance had detected 77 rabid raccoons in Lake County and 2 adjacent counties (Geauga and Cuyahoga) (Figures 1 and 2, panel A). These detections raised the

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question whether current ORV and surveillance strategies are sufficient for containment and reaching the long-term goal of regional elimination of RRV. We used molecular analyses to gain insight into the factors and possible raccoon source populations associated with the breach of the ORV zone in Ohio.

## The Study

Viral RNA was extracted as described (6) from brain tissue of 67 rabid raccoons. Samples came from raccoons in Ohio (1996 [n = 9] and 2004 [n = 10] outbreaks) and the neighboring states of Pennsylvania (2003–2004 [n = 21] and West Virginia (1987–2004 [n = 27]) (online Appendix Table, available from [www.cdc.gov/EID/content/14/4/zzz-appT.htm](http://www.cdc.gov/EID/content/14/4/zzz-appT.htm)). We amplified a 1,345-nt portion of the glycoprotein gene (G) and, for a smaller subset of samples (n = 20), the complete nucleoprotein gene (N) (1,416 nt.) (see [6] for primers and conditions). Sequences from a Florida raccoon (G, U27216; N, U27220) were included as an outgroup. When sequence data for G and N had been obtained, sequences were concatenated. After alignment, appropriate evolutionary models (7) were found for phylogenetic estimation by using maximum-likelihood and Bayesian approaches (8,9). Maximum-likelihood trees were constructed by using heuristic searches, and node support was assessed with 1,000 bootstrap replicates under the distance criterion with maximum-likelihood model settings. Bayesian estimation was performed with 2 runs of 6 million samples each and a sampling frequency of 1,000; the first 1,000 samples were discarded as burn-in.

A Bayesian molecular clock-based method (10) was used to estimate when the 2004 RRV lineage had started to diversify. To estimate evolutionary rates, we included 3 raccoon rabies sequences isolated during the larger Atlantic coast epizootic of 1982–1984. Analyses were run for 10 million steps after a burn-in period of 1 million under an exponential growth model; alternative demographic models produced equivalent results (data not shown).

According to the combined G and N data, the phylogenetic analyses showed that the 2004 Ohio outbreak was caused by a distinct RRV lineage that had limited diversity (Figure 2, panel B, red), which suggests a single-source introduction into Ohio. The 2004 lineage was not a direct descendent of any previously sampled lineages, but it shared a common ancestor with another lineage (Figure 2, panel B, yellow) that contained the viruses responsible for the 1996 Ohio outbreak along with contemporary viruses from western Pennsylvania. No members of either of these lineages had been found east of the ORV barrier (Figure 2, panel A), an area dominated by a different group of viruses (Fig-

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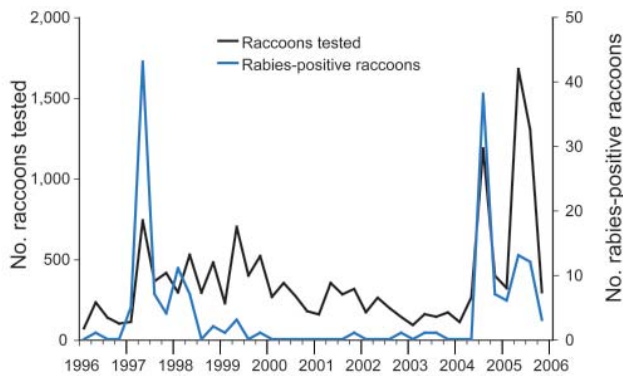


Figure 1. Raccoon rabies surveillance efforts in Ohio, 1996–2005. Data were aggregated at 3-month intervals.

ure 2, panel B, blue). The same result was obtained when the larger dataset based on G data only was analyzed (on-line Appendix Figure, available from [www.cdc.gov/EID/content/14/4/650-appG.htm](http://www.cdc.gov/EID/content/14/4/650-appG.htm)) and when we included RRV sequences from throughout eastern North America (data not shown). This finding suggests that the virus associated with the 2004 outbreak in Ohio most likely originated within the ORV zone.

Temporal estimates further indicated that all viruses sampled in the recent Ohio outbreak had started to diversify at least 3 years before 2004. The estimated dates associated with the most recent common ancestor were 1998 (highest posterior density interval 1993–2001) for the concatenated G and N data and 1995 (highest posterior density interval 1990–2000) for G data only.

**Conclusions**

Our findings imply that RRV had been circulating undetected among raccoons in the ORV zone, and possibly beyond it, for several years before its detection in 2004. These findings have important implications for the control of wildlife rabies in raccoons through ORV. First, the genetic analyses do not point to a long-distance transmission event to Ohio but rather suggest that the virus was indigenous to the region. In view of potential continued transmission events within the current ORV zone, widening the ORV corridor likely will not prevent such transmission and further spread. Second, the findings suggest that RRV may be able to persist within the ORV zone for several years and thus provide continued risk for eventual spread into unvaccinated raccoon populations. Insufficient levels of immunization among the overall population could contribute to this situation. However, spatial variation in the level of immunization or random fluctuations in the number of infected animals may also enable the virus to persist in parts of the ORV zone. Third, the

level of surveillance needed to detect RRV when transmission frequency is low is unclear.

Our results indicate that the virus had been present within Ohio for several years when surveillance efforts were relatively low; from January 2000 through June 2004, an average of 71 raccoons were tested each month compared with an average of 139 per month during 1997–1999 (Figure 1). Therefore, the critical question is: at what point would the marginal cost of increased surveillance leading to earlier detection have outweighed the cost associated with controlling the 2004 outbreak? To develop the most cost-effective strategy for containment and ultimate elimi-

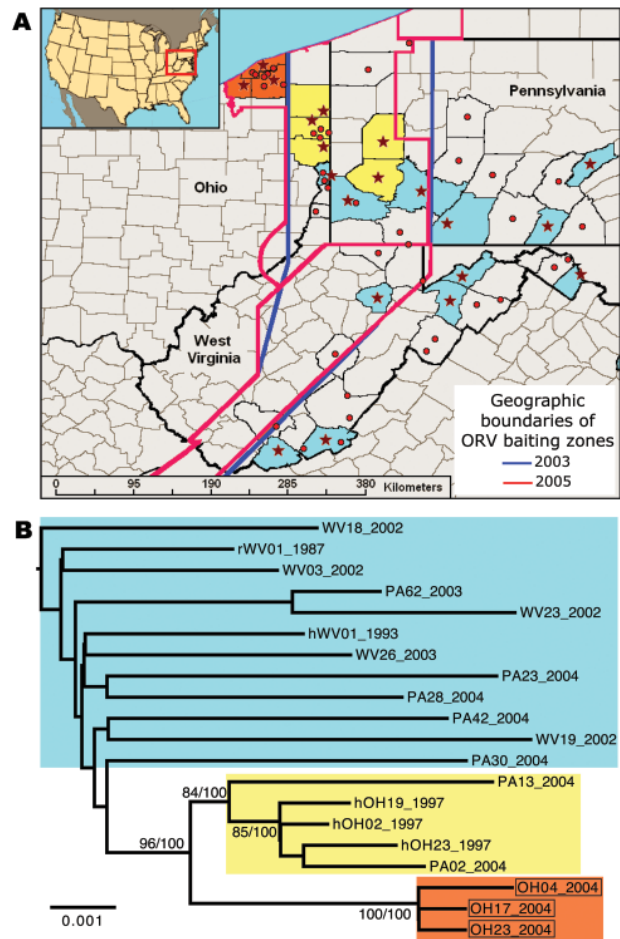


Figure 2. Spatial and genetic distribution of sequences of the raccoon rabies virus variant (RRV) from the 2004 Ohio outbreak relative to virus found in neighboring areas. A) Distribution of RRV samples included in phylogenetic analysis of G and N gene sequences (stars) or G sequences only (circles). Colors reflect phylogenetic groups as shown in panel B. B) Maximum-likelihood tree of concatenated G and N gene sequences of RRV sampled in or near Ohio, 1987–2004. Samples from the 2004 outbreak are boxed. Bootstrap values and corresponding Bayesian posterior values (% for both) are shown for key nodes. Tree was rooted by using RRV G and N sequences from a Florida raccoon (not shown). ORV, oral rabies vaccine. Scale bar = nucleotide substitutions per site.

nation of rabies among raccoons, further analyses should aim at quantifying this trade-off.

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Appendix Table. Samples and GenBank accession numbers used in this study

GenBank no.	Prep ID	SM	State	County	Date	Gene
DQ886040	hWV01N	SM1711	WV	Hancock	1993 Mar 1	N
DQ886046	PA13N	PA0407093	PA	Butler	2004 Sep 9	N
DQ886047	PA28N	PA0401719	PA	Mifflin	2004 Jul 13	N
DQ886070	rWV01N	SM1845	WV	Grant	1987 Aug 1	N
DQ886072	WV03N	WV278	WV	Monroe	2002 Aug 7	N
DQ888333	hWV01G	SM1711	WV	Hancock	1993 Mar 1	G
DQ888339	PA13G	PA0407093	PA	Butler	2004 Sep 14	G
DQ888340	PA28G	PA0401719	PA	Mifflin	2004 Jul 13	G
DQ888363	rWV01G	SM1845	WV	Grant	1987 Aug 1	G
DQ888365	WV03G	WV278	WV	Monroe	2002 Aug 7	G
DQ888367	WV08G	WV390	WV	Pendleton	2002 Aug 19	G
DQ888368	WV14G	WV465	WV	Hardy	2002 Aug 30	G
EF508133	PA23G	PA0406025	PA	Fulton	2004 Aug 26	G
EF508134	PA30G	SM6372	PA	Somerset	2004 Mar 24	G
EF508135	PA62G	3R3532	PA	Washington	2003 Apr 8	G
EF508136	WV19G	WV549	WV	Jefferson	2002 Sep 17	G
EF508137	WV23G	WV569	WV	Mineral	2002 Sep 20	G
EF508138	WV26G	SM6487	WV	Barbour	2003 Oct 29	G
EF508139	PA23N	PA0406025	PA	Fulton	2004 Aug 26	N
EF508140	PA30N	SM6372	PA	Somerset	2004 Mar 24	N
EF508141	WV19N	WV549	WV	Jefferson	2002 Sep 17	N
EF508142	WV23N	WV569	WV	Mineral	2002 Sep 20	N
EF508143	WV26N	SM6487	WV	Barbour	2003 Oct 29	N
EF508144	PA62N	3R3532	PA	Washington	2003 Apr 8	N
EU003121	hOH02N	SM3448	OH	Columbiana	1997 May 1	N
EU003122	hOH19N	SM3464	OH	Mahoning	1997 May 13	N
EU003123	hOH23N	SM3449	OH	Trumbull	1997 May 1	N
EU003124	OH04N	SM6285	OH	Cuyahoga	2004 Aug 6	N
EU003125	OH17N	SM6311	OH	Geauga	2004 Aug 30	N
EU003126	OH23N	SM6326	OH	Lake	2004 Sep 15	N
EU003127	PA02N	PA2465023_4	PA	Allegheny	2004 May 12	N
EU003128	PA42N	PA2465023_8	PA	Westmoreland	2004 May 12	N
EU003129	WV18N	WV546	WV	Mercer	2002 Sep 16	N
EU006544	hOH23G	SM3449	OH	Trumbull	1997 May 1	G
EU006545	hOH02G	SM3448	OH	Columbiana	1997 May 1	G
EU006546	hOH04G	SM3454	OH	Columbiana	1997 Apr 30	G
EU006547	hOH05G	SM3222	OH	Mahoning	1996 May 1	G
EU006548	hOH07G	SM3380	OH	Mahoning	1997 Mar 28	G
EU006549	hOH19G	SM3464	OH	Mahoning	1997 May 13	G
EU006550	hOH20G	SM3465	OH	Mahoning	1997 May 13	G
EU006551	hOH21G	SM3466	OH	Mahoning	1997 May 13	G
EU006552	OH17G	SM6311	OH	Geauga	2004 Aug 30	G
EU006553	OH01G	OH30409	OH	Lake	2004 Jul 30	G
EU006554	OH04G	SM6285	OH	Cuyahoga	2004 Aug 6	G
EU006555	OH10G	SM6291	OH	Geauga	2004 Aug 6	G
EU006556	OH11G	SM6292	OH	Geauga	2004 Aug 7	G
EU006557	OH12G	SM6294	OH	Lake	2004 Aug 9	G
EU006558	OH15G	SM6308	OH	Geauga	2004 Aug 23	G
EU006559	OH16G	SM6309	OH	Geauga	2004 Aug 27	G
EU006560	PA22G	PA0407462	PA	Franklin	2004 Sep 20	G
EU006561	OH18G	SM6311	OH	Lake	2004 Aug 30	G
EU006562	OH23G	SM6326	OH	Lake	2004 Sep 15	G
EU006563	PA02G	PA2465023_4	PA	Allegheny	2004 May 12	G
EU006564	PA04G	PA0402143_3	PA	Allegheny	2004 Jul 16	G
EU006565	PA10G	SM6371	PA	Bedford	2004 Mar 7	G
EU006566	PA15G	PA0406341	PA	Cambria	2004 Aug 31	G
EU006567	PA19G	SM6377	PA	Fayette	2004 Aug 30	G
EU006568	PA55G	3R3513	PA	Crawford	2003 Apr 11	G
EU006569	PA25G	PA0405472	PA	Huntingdon	2004 Aug 19	G
EU006570	PA26G	PA0400421_9	PA	Indiana	2004 Jun 28	G
EU006571	PA27G	PA0407458	PA	Jefferson	2004 Sep 20	G
EU006572	PA32G	PA0401161	PA	Washington	2004 Jul 8	G
EU006573	PA33G	PA0402026_2	PA	Washington	2004 Jul 15	G
EU006574	PA38G	SM6376	PA	Westmoreland	2004 Apr 13	G
EU006575	PA42G	PA2465023_8	PA	Westmoreland	2004 May 12	G
EU006576	WV16G	WV531	WV	Greenbrier	2002 Sep 12	G
EU006577	PA57G	3R3717	PA	Erie	2003 May 6	G
EU006578	PA58G	3R4497	PA	Fayette	2004 Jul 2	G

EU006579	WV01G	WV210	WV	Raleigh	2002 Jul 30	G
EU006580	WV06G	WV384	WV	Hardy	2002 Aug 19	G
EU006581	WV07G	WV385	WV	Berkeley	2002 Aug 19	G
EU006582	WV09G	WV400	WV	Hancock	2002 Aug 20	G
EU006583	WV11G	WV410	WV	Webster	2002 Aug 21	G
EU006584	WV30G	SM5998	WV	Hancock	2003 Jul 16	G
EU006585	WV18G	WV546	WV	Mercer	2002 Sep 16	G
EU006586	WV21G	WV552	WV	Pendleton	2002 Sep 17	G
EU006587	WV24G	WV581	WV	Monongalia	2002 Sep 24	G
EU006588	WV25G	WV1014	WV	Berkeley	2002 Dec 23	G
EU006589	WV27G	SM6483	WV	Brooke	2003 Sep 10	G
EU006590	WV28G	SM5995	WV	Greenbrier	2003 Jul 1	G
EU006591	WV48G	SM6002	WV	Preston	2003 Aug 8	G
EU006592	WV37G	SM6484	WV	Monongalia	2003 Sep 15	G
EU006593	WV40G	SM5993	WV	Monroe	2003 Jun 19	G
EU006594	WV42G	SM6489	WV	Monroe	2004 Feb 10	G
EU006595	WV45G	SM5977	WV	Ohio	2003 Mar 11	G
EU006596	WV46G	SM5980	WV	Ohio	2003 Apr 3	G
EU006597	WV47G	SM5973	WV	Preston	2003 Feb 4	G