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Evaluation of species identification and rabies virus characterization among bat rabies cases in the United States

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Abstract

OBJECTIVE—To evaluate species identification and rabies virus (RABV) characterization among samples from bats submitted for rabies testing in the United States and assess whether a standardized approach to specimen selection for RABV characterization could enhance detection of a sentinel event in virus dissemination among bats.

SAMPLE—United States public health rabies surveillance system data collected in January 2010 through December 2015.

PROCEDURES—The number of rabies-tested bats for which species was reported and the number of RABV-positive samples for which virus characterization would likely provide

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information regarding introduction of novel RABV variants and translocation and host-shift events were calculated. These specimens were designated as specimens of epizootiological importance (SEIs). Additionally, the estimated test load that public health laboratories could expect if all SEIs underwent RABV characterization was determined.

RESULTS—Species was reported for 74,928 of 160,017 (47%) bats submitted for rabies testing. Identified SEIs were grouped in 3 subcategories, namely nonindigenous bats; bats in southern border states, Florida, Puerto Rico, and the US Virgin Islands; and bats of species that are not commonly found to be infected with RABV. Annually, 692 (95% CI, 600 to 784) SEIs were identified, of which only 295 (95% CI, 148 to 442) underwent virus characterization. Virus characterization of all SEIs would be expected to increase public health laboratories' overall test load by 397 (95% CI, 287 to 506) samples each year.

CONCLUSIONS AND CLINICAL RELEVANCE—Species identification and RABV characterization may aid detection of a sentinel event in bat RABV dissemination. With additional resources, RABV characterization of all SEIs as a standardized approach to testing could contribute to knowledge of circulating bat RABV variants.

Rabies virus (RABV) is a member of the genus *Lyssavirus* within the family Rhabdoviridae. At least 16 distinct *Lyssavirus* spp have been formally recognized, 10 of which have been isolated from multiple bats species, and all appear to cause the disease known as rabies.[1] Rabies virus infection results in a fatal encephalitic illness in mammals. Although any mammal can be infected with RABV, there are specific reservoir species that are associated with enzootic transmission. In the continental United States and US Caribbean territories (Puerto Rico and US Virgin Islands), RABV reservoir species include striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), Arctic foxes (*Vulpes lagopus*), gray foxes (*Urocyon cinereoargenteus*), small Indian mongooses (*Herpestes auropunctatus*), and a diverse assemblage of bats (order Chiroptera). Rabies virus variants are transmitted primarily within a single reservoir species, although cross-species transmission can occur.[2]

Among humans with rabies acquired in the United States, bat RABV variants account for most deaths[3]; thus, rabies in bats is a major public health concern. The RABVs associated with silver-haired bats (*Lasionycteris noctivagans*), Brazilian free-tailed bats (*Tadarida brasiliensis*), and tricolored bats (*Perimyotis subflavus*) are most commonly associated with human RABV infections.[2,4] However, silver-haired and tricolored bats are not often found in human habitats, and there is limited knowledge of the RABV variants circulating in these inconspicuous bat species populations.[2,5,6] In contrast, big brown bats (*Eptesicus fuscus*), little brown bats (*Myotis lucifugus*), and Brazilian free-tailed bats are conspicuous species that roost frequently in human-made structures, increasing the likelihood of contact with humans and domestic animals.[7-9] As a result, these bat species are more commonly tested for rabies in public health laboratories.

The circulation of RABV in bats is unique to the western hemisphere.[10] However, the epizootiology of bat rabies as a whole is similar to the perpetuation of RABV variants among carnivore species.[11] There are approximately 60 bat species indigenous to the continental United States,[12-14] Puerto Rico, and the US Virgin Islands, of which at least 31 have been reported to be infected with RABV.[2,5,15,16] There are more than 20 RABV variant phylogenetic lineages described for bat species in the United States, and most are associated with individual species.[2,17] Bat RABV variants may cause infection in other susceptible bat hosts, most often causing dead-end rabies spillover infections as observed for carnivore RABV variants. Multiple RABV variants may be associated with a given bat species,[17-19] and transmission dynamics may be shaped by the frequency of interspecies contacts resulting from geographic range overlap, foraging, and roosting behaviors.[17] The genetic similarity among bat species, as well as their geographic overlap, may be a major influence on RABV host-shift events among bats.[11,20] Bat-associated RABV host shifts to terrestrial mammals have been implicated in epizootics among gray fox and striped skunk populations in the south-western United States[20,21] and are responsible for at least 1 historic shift of RABV into carnivores in the United States.[20] Compared with carnivore reservoir species, less is known about RABV circulation in bat species in the United States.[22] Given the impacts that bat RABV has on public health and the role of bat RABV in host-shift events, understanding the transmission dynamics of RABV variants within and among bat species is crucial.

The introduction into the United States of RABV variants associated with nonindigenous bat species, such as the common vampire bat (*Desmodus rotundus*) and the hairy-legged vampire bat (*Dipbylla ecaudata*), is also a risk. The circulation of RABV among common vampire bats causes considerable economic and livestock losses throughout the geographic range of this bat species.[23-25] Surveillance along the United States-Mexico border is essential to detect the potential introduction of nonindigenous bat species.[26] Furthermore, in the event that nonindigenous bat species do establish colonies within the United States, local identification of novel RABV variants may serve as a sentinel signal for novel host species introduction. A timely public and animal health response to novel RABV variant introductions would minimize the risk of exposure of humans and animals and protect livestock from an incursion of rabies with potentially severe economic impacts.

Animal rabies is a notifiable event in the United States.[2,5] Currently, national rabies testing is primarily performed by state public health and veterinary diagnostic laboratories. Each year, state health departments and the USDA APHIS Wildlife Services’ National Rabies Management Program submit data to the national RSS, which is maintained by the Poxvirus and Rabies Branch at the CDC. In some states, additional laboratory analysis to determine the RABV variant, referred to as virus characterization, can be performed. The CDC serves as the national reference laboratory in the United States.

The purpose of the study of this report was to assess the frequency with which bat species are identified when specimens are submitted for rabies testing, determine which rabid bat specimens merit prioritization for virus characterization, and estimate the additional testing load for public health laboratories if all prioritized specimens underwent RABV characterization.

Materials and Methods

SEI selection criteria

Rabies surveillance data for bats in the United States that were submitted to the CDC during the period of January 1, 2010, through December 31, 2015, were included in this evaluation. Terrestrial nonvolant mammal data were not included but underwent separate analysis.[27] The RSS data were reviewed to identify RABV-positive bat specimens for which virus characterization could help detect sentinel events in virus dissemination and dispersal. The 2 sentinel events of interest included the early detection of a host-shift event and translocation of a bat RABV variant or the introduction of an RABV variant to a new area. Rabies virus-positive specimens most likely to aid in detection of such an event were referred to as SEIs. These SEIs were subcategorized into 1 of 3 groups as follows: nonindigenous rabid bat species; rabid bats in southern border states (Arizona, California, New Mexico, and Texas), Florida, Puerto Rico, and the US Virgin Islands; and rabid bats that are not commonly found to be infected with RABV. Rabid bats not commonly found to be infected with RABV included all indigenous bat species other than the 5 most common rabid bat species (big brown bat [*E fuscus*], little brown bat [*M lucifugus*], Brazilian free-tailed bat [*T brasiliensis*], hoary bat [*Lasiurus cinereus*], and eastern red bat [*Lasiurus borealis*]).

Databases and analyses

State health departments do not typically report the virus characterization method applied by the public health laboratories; therefore, for the purposes of this report, the term RABV characterization refers to either antigenic or nucleotide-based sequencing methods.[28,29] Bat species identification was performed by the state public health and veterinary diagnostic laboratories or the CDC.

Data were analyzed with commercially available software[a] and statistical programs.[b],[c] Percentages and descriptive statistics (with 95% CIs) are reported. The total number of RABV-positive samples in each SEI subcategory across all 6 years was used to calculate the estimated number of additional samples (and corresponding CIs) that public health laboratories could expect to process if all SEIs underwent RABV characterization. Some SEIs could be placed in multiple subcategories; however, duplicate samples from multiple SEI subcategories were removed to calculate the total sample increase expected by public health laboratories. Sample size and proportional frequencies were calculated with 99% CIs to assess the number of samples that would need to undergo RABV characterization to detect a novel variant or a difference in variants.

Bat variants in this analysis included those associated with species of *Eptesicus* (*E fuscus*), *Lasionycteris* (*L noctivagans*), *Lasiurus* (*L borealis*, *L cinereus*, *Lasiurus ega*, *Lasiurus intermedius*, and *Lasiurus xanthinus*), *Myotis* (*M lucifugus* and *Myotis velifer*), *Nycticeius* (*Nycticeius humeralis*), *Parastrellus* (*Parastrellus hesperus*), *Perimyotis* (*P subflavus*), and

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[a] Microsoft Access 2013, Microsoft Corp, Redmond, Wash.
[b] STATA 13.1, StataCorp LLC, College Station, Tex.
[c] OpenEpi: Open Source Epidemiologic Statistics for Public Health, version 3.01. Available at: www.OpenEpi.com. Accessed Oct 1, 2016.

Tadarida (*T brasiliensis*). There are additional bat variants present throughout the United States; however, the aforementioned variants were the only variants reported to the US national RSS during the 2010–2015 period.

Results

State health department and US territory health department bat data

All state health departments and the New York City health department received bats for rabies testing during 2010 through 2015. Hawaii was the only state that did not receive bats for rabies testing. Although Hawaii has 1 native bat species (*L cinereus*), rabies is not present in Hawaii. Nine state health departments did not report any bat species data, and 26 state health departments did not report RABV characterization data; however, RABV variant reporting is not an explicit requirement on the basis of recommendations of the Council of State and Territorial Epidemiologists regarding national reporting. Ten (19.6%) state health departments identified bat species and reported RABV characterization data to the national rabies program. Thirty-two (62.7%) state health departments identified bat species but did not report RABV characterization data or reported characterization results for < 10% of rabid bats. Twelve (23.5%) health departments identified the species of < 10% of bat submissions. Nine (17.6%) state health departments did not routinely report either bat species or RABV characterization data.

United States territories reported limited bat data to the US national RSS over the 6-year period; Puerto Rico provided data for 15 samples, and the US Virgin Islands provided data for 1 sample. Although US territories may collect and test bats suspected of being infected with RABV, there are minimal data submitted to the US national RSS.

Species identification of bats

During 2010 through 2015, there were 160,017 bats submitted for testing, of which 9,552 (6.0%) were RABV positive (Table 1). Among the RABV-positive specimens, 2,029 (21.2%) were characterized to identify the RABV variant. There were 74,928 (46.8%) bats for which species was identified among all submissions; 4,046 (5.4%) of those species-identified bats were RABV positive, of which 1,157 (28.6%) specimens underwent RABV characterization (Table 2). Among the 160,017 bats submitted for testing, there were 85,089 (53.2%) specimens for which species was not identified (identification to order or genus only). Of the 85,089 species-unidentified bats tested for rabies, 5,506 (6.5%) were RABV positive; 909 (16.5%) specimens underwent RABV characterization. The mean annual number of bats that were submitted for RABV testing but were not identified to the species level was 14,181 (95% CI, 13,384 to 14,978). Annually, the mean number of RABV-positive bat specimens for which species was identified was 674 (95% CI, 467 to 881), and the mean number of RABV-positive bat specimens for which species was not identified was 917 (95% CI, 746 to 1,088).

During the 2010–2015 period, specimens from 38 of the approximately 60 bat species (submissions ranged from 1 to 62,997 specimens/species) indigenous to the continental United States, Puerto Rico, and the US Virgin Islands were submitted for RABV testing, and at least 1 rabid bat (range, 1 to 2,269 specimens/species) was detected in 27 of the 38 species submitted. Specimens of 5 nonindigenous species (n = 27; range, 1 to 16 specimens/species) were submitted for testing; all those bats were negative for RABV. The nonindigenous species were submitted by zoos and research facilities and did not represent natural introduction events involving nonindigenous bat species.

Identification of SEIs

Rabies virus–positive bat specimens most likely to aid in detection of the 2 sentinel events of interest (ie, early detection of a host-shift event and translocation of a bat RABV variant or the introduction of an RABV variant [focused on the common vampire bat {*D rotundus*} RABV variant] to a new area) were identified. These SEIs were subcategorized into 1 of 3 groups as follows: nonindigenous rabid bat species; rabid bats in southern border states (Arizona, California, New Mexico, and Texas), Florida, Puerto Rico, and the US Virgin Islands; and rabid bats of species that are not commonly found to be infected with RABV.

Of the 160,017 bats submitted for testing during 2010 through 2015, 29,988 (18.7%) were collected in southern border states (Arizona, California, New Mexico, and Texas), Florida, Puerto Rico, and the US Virgin Islands. Of the 29,988 bat specimens from these regions, 3,974 were RABV positive and selected as SEIs. Among the 74,928 bats submitted for rabies testing for which species was identified, 27 (0.02%) were nonindigenous bat species, and 3,867 (2.4%) were bats that are not commonly found to be infected with rabies. Among the nonindigenous bat specimens, 0 were RABV positive. Among the specimens of bats that are not commonly found to be infected with rabies, 292 were RABV positive and selected as SEIs. The mean number of rabid bats submitted annually for rabies testing in the United States during 2010 through 2015 was 1,592 (95% CI, 1,430 to 1,753), of which 692 (95% CI, 600 to 784) were considered SEIs.

Virus characterization of SEIs

There were 2,029 rabid bat samples that underwent virus characterization. Of those, 126 (6.2%) specimens had the variant identified as bat, bat resident strain, bat NOS (not otherwise specified), or bat migratory species in the data set. These classifications were not informative and were removed during the viral characterization analysis.

During January 2010 through December 2015, 1,903 specimens (excluding the aforementioned 126 specimens) underwent virus characterization, and 7,523 specimens did not undergo virus characterization. The mean annual number of SEIs that underwent virus characterization was 295 (range of mean annual number of specimens characterized, 148 to 442). The mean annual number of SEIs that did not undergo virus characterization was 397.

Subcategories of SEIs that would identify or provide information regarding detection of the introduction of novel variants and translocation events

Two subcategories of SEIs were found to provide the most meaningful information regarding detection of the introduction of novel variants (focused on the common vampire bat [*D rotundus*] RABV variant) and translocation events. Among the SEIs, those from bats collected in southern border states (Arizona, California, New Mexico, and Texas), Florida, Puerto Rico, and the US Virgin Islands were considered prime candidates for RABV characterization.

During the 2010–2015 period, 29,988 bats were submitted for rabies testing from the southern border states, Florida, Puerto Rico, and the US Virgin Islands (Table 3). Among the bats submitted, 3,974 (13.3%) were rabid; 1,765 (45.6%) of those specimens underwent virus characterization. The mean annual number of SEIs from those states and US territories was 662 (95% CI, 572 to 752), of which 294 (45.6%; 95% CI, 146 to 441) did and 368 (95% CI, 251 to 484) did not undergo virus characterization. The RABV variants detected included *T brasiliensis* (1,531 SEIs [86.7%]), *L borealis* (77 SEIs [4.3%]), *N humeralis* (45 SEIs [2.5%]), *L. cinereus* (31 SEIs [17%]), *L. intermedius* (25 SEIs [1.4%]), *P hesperus* (19 SEIs [1.1%]), *E fuscus* (19 SEIs [1.1%]), *L xanthinus* (7 SEIs [0.3%]), *L ega* (5 SEIs [0.28%]), *M velifer* (3 SEIs [0.16%]), *P subflavus* (2 SEIs [0.11%]), and *L noctivagans* (1 SEI [0.05%]).

The other SEI subcategory considered likely to provide information regarding detection of the introduction of novel variants and translocation events was nonindigenous bat species. Of the 74,928 bats for which the species was identified, 27 (0.04%) nonindigenous bats were identified and underwent rabies testing; all those bats were RABV negative. Nonindigenous species included the Egyptian fruit bat (*Rousettus aegyptiacus* [n = 15]), Indian flying fox (*Pteropus giganteus* [5]), common vampire bat (*D rotundus* [3]), long-haired rousette (*Rousettus lanosus* [2]), and black mastiff bat (*Molossus rufus* [1]). All nonindigenous bat specimens submitted for testing were associated with zoos and research facilities.

Among the 160,017 bats submitted for RABV testing, 85,089 (53.2%) were not identified at the species level. However, virus characterization of RABV-infected nonindigenous bats would be unlikely to result in additional specimens on the basis of the analysis of current surveillance data, assuming prevalence rates remained relatively constant.

Subcategories of SEIs that would identify or provide information regarding early detection of a host-shift event

The subcategory of SEIs considered most likely to provide the most meaningful information regarding a host-shift event was detection of RABV in bats of species that are not commonly found to be infected with RABV.

The data analyzed in this subcategory excluded data from those specimens obtained from bats in southern border states (Arizona, California, New Mexico, and Texas), Florida, Puerto Rico, and the US Virgin Islands. Among the bats submitted for RABV testing during 2010 through 2015, the 5 most common bat species (big brown bat [*E fuscus*], little brown bat [*M lucifugus*], Brazilian free-tailed bat [*T brasiliensis*], hoary bat [*L cinereus*], and eastern red bat [*L borealis*]) and all nonindigenous bats accounted for 3,867 of the tested specimens. Of those 3,867 specimens, 292 (7.6%) were RABV positive; 62 (21.2%) of the RABV-positive specimens underwent virus characterization. The mean annual number of rabid bats that are not commonly found to be infected with RABV was 48 (95% CI, 28 to 69), of which 10 (95% CI, 0 to 22) underwent virus characterization.

Overall impact of testing all SEIs for public health laboratories

Virus characterization of all RABV-positive SEIs would result in an estimated increase in public health laboratories' test load of 397 (95% CI, 287 to 506) samples nationwide each year; however, a mean of 368 (95% CI, 251 to 484) specimens would be expected to originate from Arizona, California, New Mexico, Texas, Florida, Puerto Rico, and the US Virgin Islands (Table 3). These estimates do not account for unidentified, lesser-known bat species outside southern border states, Florida, Puerto Rico, and the US Virgin Islands because the true number of SEIs was not known owing to missing species data. Assuming similar species distribution patterns (5.1% of identified bat species were bat species that are not commonly found to be infected with RABV) among the annual 472 (95% CI, 421 to 524) RABV-positive samples that were not identified by species and were derived from bats located outside southern border states, Florida, Puerto Rico, and the US Virgin Islands, the mean estimated number of additional lesser-known bat species' SEIs requiring RABV characterization each year would be 23 (95% CI, 20 to 25).

Data for bats that are commonly infected with RABV and testing considerations

The 5 bat species in the United States that are most commonly RABV positive are the big brown bat (*E fuscus*), little brown bat (*M lucifugus*), Brazilian free-tailed bat (*T brasiliensis*), hoary bat (*L cinereus)*, and eastern red bat (*L borealis*). In the present evaluation, *T brasiliensis, E fuscus*, and *L borealis* were frequently infected with species-specific variants (Table 4). Low numbers of specimens of *M lucifugus* and *L cinereus* underwent RABV characterization, thereby making it difficult to assess how frequently these bat species were infected with species-specific RABV variants. During 2010 through 2015, there was a sufficient number of *T brasiliensis* specimens tested to detect differences in variants among the RABV-positive specimens. However, insufficient numbers of RABV-positive specimens of *E fuscus, L borealis*, *L cinereus*, and *M lucifugus* were characterized for similar analyses.

Discussion

Results of the analysis of RSS data for bats in the United States that were submitted to the CDC during the period of January 2010 through December 2015 revealed that every year, the species of more than half (53%) of the bats submitted for rabies testing was not identified. Although there are limits to testing capabilities, state and territorial health departments should attempt to identify the species of submitted bat specimens to contribute to the understanding of bat rabies dynamics at the species level. Species-level identification of rabid bats is critical to document important reservoir hosts, provide information regarding the spatial-temporal dynamics of spillover infections among different bat species,[28] and identify the introduction of nonindigenous bat species into the United States. Both the USDA and CDC monitor for the introduction of foreign animal diseases, especially those that are zoonotic and may pose a substantial economic burden if they become enzootic in the United States. The US national RSS is one of the largest animal disease databases in the United States, and data supplied by state and territorial health departments help inform the CDC and USDA of issues of national concern (ie, importation or migration of nonindigenous species).

To assist all jurisdictions in identifying bat species, improved technology and better guidance may be needed. Many bats, particularly *Myotis* spp, can be difficult to identify and distinguish from other morphologically similar species.[30] Improved identification tools such as genetic barcoding may provide useful information in cases of specimen degradation.[17,30-33]

Molecular characterization of bat RABV variants provides information regarding the transmission dynamics of those variants because antigenic typing is often not sufficiently sensitive to distinguish bat RABV variants.[28] In the present evaluation, 9 state health departments did not report any bat species data, and 26 state health departments did not report RABV characterization data. The CDC requests characterization information be provided, but on a voluntary basis. The lack of species identification and RABV characterization data may have reflected a lack of voluntary reporting of completed tests to the national RSS; however, it may also indicate potential deficiencies in laboratory training, staff shortages, unfulfilled equipment needs, or a lack of funding or awareness of the value of this information in the control and prevention of rabies.

On the basis of the data assessed in the present evaluation, characterization of RABV in all SEIs (as defined by the study criteria) would result in a mean estimated nationwide increase of 397 (95% CI, 287 to 506) specimen/y; of those additional specimens, 368 (95% CI, 251 to 484) specimens would be expected to originate from Arizona, California, New Mexico, Texas, Florida, Puerto Rico, and the US Virgin Islands. Most of the bat specimens classified as SEIs in the present evaluation originated from 5 states (Arizona, California, New Mexico, Texas, and Florida), which reflected a disproportionate burden on a small number of states. All RABV-positive bat specimens obtained in Arizona, California, New Mexico, Texas, Florida, Puerto Rico, and the US Virgin Islands should undergo RABV characterization and be stored (Appendix) as part of a robust surveillance system to monitor for the introduction of novel variants. The southern portion of the United States and the Caribbean contain a broad variety of bat species. Testing of a larger number of specimens from these geographic regions would provide enhanced insight into the current diversity of bat species and the RABV variants circulating within those bat populations, facilitate monitoring of RABV ecology and evolution in these areas, and improve understanding of the geographic and temporal RABV transmission dynamics among bat species in the regions. However, an increased test load in these areas should be balanced with available health departments' resources. Partnerships of public health departments with universities, other government agencies, and private research collaborators may offset costs while enabling enhancement of knowledge of bat species and bat RABV variants in a given region.

The 5 bat species most commonly infected with RABV have been associated with specific RABV variants that circulate enzootically within their populations, although data are still sparse. Among rabid *T brasiliensis* across the entire United States and Mexico, there is a high degree of RABV homogeneity.[20] Among rabid *T brasiliensis* in the United States, 99.6% are infected with the *T brasiliensis* RABV variant. Although RABV variant homogeneity among *L borealis* and *L cinereus* is also high, continued virus characterization is recommended for these species as well as for *E fuscus* and *M lucifugus* until more data are obtained. Additionally, RABV characterization of RABV-positive specimens from these bat species should always be performed when the bats are found in geographic regions of interest (California, Arizona, New Mexico, Texas, Florida, Puerto Rico, and the US Virgin Islands) to monitor for introduction of novel viruses, such as the *D rotundus* RABV variant. Furthermore, focusing limited public health resources on identification and characterization of lesser-known bat species, some of which are known to have frequent human contact, would likely provide more valuable information on bat species and RABV ecology and disease transmission dynamics.

There were several limitations to the present evaluation. First, limited data from US territories were reported, with fewer than 20 samples being submitted for testing over the 6-year period. Bats from the US Virgin Islands and Puerto Rico could potentially travel to nearby areas of the continental United States, such as Florida. Having a thorough understanding of whether RABV variants are circulating in bat populations in the Caribbean as well as knowledge of which RABV variants are present may influence surveillance and rabies prevention and control activities in the United States and import and travel recommendations. Second, multiple *E fuscus* RABV variants were combined and analyzed as a single variant, even though there are several *E fuscus* variants circulating in big brown bat populations in the United States.[17,20] Without a standard approach to defining and reporting RABV variants, there will continue to be confusion and potential misinterpretation of bat rabies surveillance data. Finally, state health departments and public health laboratories often send RABV-negative bats to universities for species identification; however, RABV-positive bats are not permitted to leave the laboratory. This makes it less likely that rabid bats will be identified and highlights the need for public health laboratories to have access to bat species identification training through genetic barcoding,[31] application of online taxonomic tools, or published guides for quick and easy reference.[32,33]

Rabies virus variant characterization data are unlikely to impact postexposure prophylaxis recommendations for persons exposed to rabid bats; however, there is the potential for importation of bats or foreign bat lyssaviruses into the United States. Ideally, the national RSS could be used to detect outbreaks in bat populations, distinguish atypical variant circulations in bat species, understand potential seasonal patterns of dispersal, identify epidemiologic patterns of infection, and send out public health alerts when necessary. Electronic real-time reporting, which is currently being piloted in several states, is a crucial component of real-time rabies surveillance feedback. However, this information would be of limited value without a thorough understanding of bat species and the RABV variants that circulate among them. The rabies vaccine does not confer cross-protection to all lyssaviruses; therefore, it would also be prudent to enhance the understanding of non-RABV lyssavirus variants for public health situational awareness.[34-36] Although the US public health system would benefit from this knowledge, there is an associated cost that should be carefully considered when determining future testing recommendations. However, the fact that human deaths associated with bat RABV infection continue to occur in the United States highlights that additional effort to characterize trends in bat RABV transmission dynamics is warranted.

The success of the RSS among states requires collaboration among epidemiologists, wildlife biologists, and personnel working in animal control programs and public health laboratories. However, the infrastructure, resources, and surveillance systems of each state differ, requiring each state to independently evaluate its current surveillance system and determine how to incorporate the proposed SEI criteria for specimen testing into current laboratory practices. The need to characterize RABV variants in rabid bats may change over time with improved ability to identify bat species and the RABV variants that circulate within those species. To date, far less is known about the epidemiology of bat RABV variants circulating in the United States than that of carnivore RABV variants. Enhancement of knowledge of circulating bat RABV variants will require more comprehensive sample analysis over an extended period than what is needed to address data deficiencies for carnivore reservoirs. Cost-benefit analysis and refinement to optimize surveillance systems would ultimately provide more detail to better target appropriate sample sizes for testing and subsequent detection of RABV variants of public health importance.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC or the USDA.

Appendix

Recommendations for storage of specimens obtained from rabid bats.

Suggested duration of storage

Any RABV-positive specimen that undergoes virus characterization should be retained for long-term storage.

Suggested preservation methods

Fresh, well-preserved cross sections of brainstem containing 3+ or 4+ intensity of fluorescein isothiocyanate-labeled antibody against RABV should be stored long term. The ideal amount of tissue to freeze and store is the amount of cross-sectioned brainstem or whole bat brain that can be fitted in half of the capacity of a 2-mL cryotube (with an O-ring cap assembly). Samples submitted in tin or large containers (volume, > 2 mL) should be aliquoted in 2-mL cryotubes after an adequate 3+ or 4+ section (as determined by direct fluorescent antibody testing) was selected.

ABBREVIATIONS

CI	Confidence interval
RABV	Rabies virus
RSS	Rabies surveillance system
SEI	Specimen of epizootiological importance

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Table 1—

Number of bats tested for rabies and number of specimens positive for RABV, by species or group, in the United States during the period of January 2010 through December 2015.

Species or group	**No. of bats tested for rabies**	**No. of RABV-positive specimens (%)**
Unidentified	85,089	5,506 (6.5)
Big brown bat (*Eptesicus fuscus*)	62,997	2,269 (3.6)
Little brown bat (*Myotis lucifugus)*	4,560	118 (2.6)
Brazilian free-tailed bat (*Tadarida brasiliensis*)	2,006	1,050 (52.3)
Other	3,894	292 (7.5)
Eastern red bat (*Lasiurus borealis*)	1,162	175 (15.1)
Hoary bat (*Lasiurus cinereus*)	309	142 (46.0)
All bats	**160,017**	**9,552 (6.0)**

Table 2—

Data regarding bat specimens tested for rabies that were or were not identified to the species level by year in the United States during January 2010 through December 2015.

Variable	Year						Mean (95% CI)
	2010	2011	2012	2013	2014	2015	
Total No. of bats submitted	25,194	24,583	27,649	25,548	29,689	27,354	26,669 (24,659–28,679)
Total No. of RABV-positive specimens (%)	1,430 (5.7)	1,380 (5.6)	1,680 (6.1)	1,602 (6.3)	1,756 (5.9)	1,704 (6.2)	1,592 (1,430–1,753)
Bat species not identified							
No. of specimens tested (% of total No. of specimens tested)	13,768 (54.6)	14,266 (58.0)	13,959 (50.5)	13,150 (51.5)	15,383 (51.8)	14,563 (53.2)	14,181 (13,384–14,978)
No. of RABV-positive specimens (%)	657 (4.8)	966 (6.8)	1,055 (7.6)	1,106 (8.4)	828 (5.4)	894 (6.1)	917 (746–1,088)
No. of RABV-positive specimens that underwent virus characterization (%)	31 (4.7)	53 (5.5)	360 (34.1)	435 (39.3)	14 (1.7)	16 (1.8)	151 (0–353)
Bat species identified							
No. of specimens tested (% total No. of specimens tested)	11,426 (45.3)	10,317 (42.0)	13,690 (49.5)	12,397 (48.5)	14,305 (48.2)	12,790 (46.8)	12,487 (10,953–14,021)
No. of RABV-positive specimens (%)	773 (6.7)	414 (4.0)	625 (4.6)	496 (4.0)	928 (6.5)	810 (6.3)	674 (467–881)
No. of RABV-positive specimens that underwent virus characterization (%)	341 (44.1)	30 (7.2)	30 (4.8)	28 (5.6)	420 (45.2)	308 (38.0)	192 (1–384)

Table 3—

Number of RABV-positive bat specimens considered to be SEIs and number of SEIs that underwent RABV characterization in the United States during the period of January 2010 through December 2015, and estimated increase in public laboratories' test load if all SEIs were to undergo viral characterization.

Sentinel event	SEI subcategory	Mean (95% CI) annual No. of SEIs	Mean (95% CI) annual No. of SEIs that underwent virus characterization	Mean (95% CI) annual No. of additional specimens processed if all SEIs were to undergo RABV characterization
Translocation of a bat RABV variant or the introduction of an RABV variant to a new area	Bats in Arizona, California, New Mexico, Texas, Puerto Rico, and the US Virgin Islands	662 (572–752)	294 (22–403)	368 (251–484)
	Nonindigenous bat species	0	0	Not estimated
Host-shift event	Bats not commonly found to be infected with RABV*	30 (23–36)	1 (0–2)	29 (21–36)
Total		**692 (600–784)**	**295 (148–442)**	**397 (287–506)**

Rabies virus-positive specimens most likely to aid in detection of such a sentinel event were classified as SEIs.

*Grouping excluded bats from Arizona, California, New Mexico, Texas, and US territories.

Table 4—

Rabies virus testing data for the 5 bat species most commonly infected with RABV in the United States during the period of January 2010 through December 2015.

Species	No. (%) of RABV-positive samples	No. (%) of RABV-positive specimens that underwent virus characterization	Percentage (95% CI) of RABV-positive specimens characterized as reservoir species-specific variant	Mean (95% CI) annual No. of RABV-positive samples	Mean (95% CI) annual No. of RABV-positive samples that underwent RABV characterization
Brazilian free-tailed bat (*Tadarida brasiliensis*)	1,050 (52.3)	890[*] (84.7)	99.6 (99.0–99.9)	175 (0.6–349)	148 (0–323)
Big brown bat (*Eptesicus fuscus*)	2,269 (3.6)	116 (5.1)	94.8 (89.1–98.1)	378 (316–440)	19 (9–30)
Eastern red bat (*Lasiurus borealis*)	175 (15)	43 (24.6)	97.7 (87.7–99.9)	29 (15–42)	7 (0.6–13)
Hoary bat (*Lasiurus cinereus*)	142 (45.9)	17 (11.9)	94.1 (71.3–99.8)	23 (15–31)	3 (0–6)
Little brown bat (*Myotis lucifugus*)	118 (2.5)	7 (5.9)	42.8 (9.9–81.6)	19 (14–25)	1 (0–2)

[*] The number of RABV-positive specimens that underwent virus characterization was sufficient to detect a difference in variants in the sample population with 99% confidence.