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# Diagnostic sensitivity of alternative samples and tissues for *Lyssavirus rabies* detection in striped skunks (*Mephitis mephitis*)

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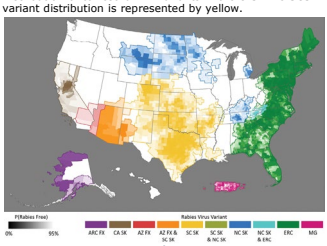
## INTRODUCTION

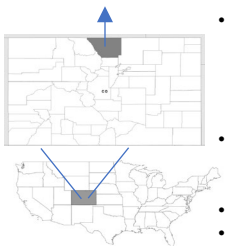
- Enhanced rabies surveillance (ERS) is designed to improve detection of wildlife *Lyssavirus rabies* (RV) variants by supplementing national public health surveillance with targeted sampling, particularly among strange acting animals.
- A sensitive and specific RV diagnosis is ideal for ERS.
- RNA degradation and cold storage requirements can complicate field collection of diagnostic samples for RV detection.
- Objective: Evaluate rtRT-PCR on non-CNS samples from striped skunks (*Mephitis mephitis*) infected with the southcentral skunk variant of RV (SCSK) to determine the utility of alternative, field-friendly diagnostic samples for ERS.

Distribution of terrestrial RV variants in the U.S. The SCSK variant distribution is represented by yellow.



Ma et al. 2024[1]

## METHODS

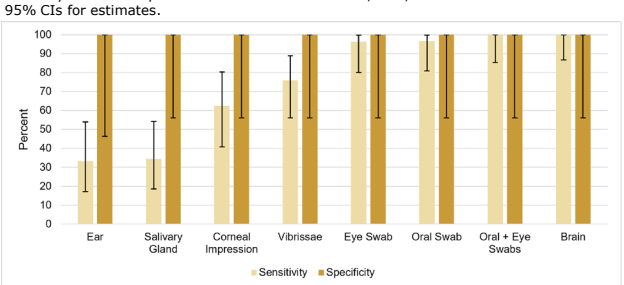
- Samples were opportunistically collected from 41 striped skunks submitted for public health and enhanced surveillance during 2019 from a SCSK RV enzootic area in northern Colorado, USA.
  - Brain, corneal impression, ear, eye swab, oral swab, salivary gland, and vibrissae were collected from each skunk.
- Rabies diagnosis with brain tissue was performed by the Colorado State University Veterinary Diagnostic Laboratory.
  - We sampled 34 RV positive and 7 negative skunks
- Total RNA was extracted using Zymo Research Quick-DNA/RNA MiniPrep Plus Kit
- Each sample was evaluated in triplicate by modified LN34 rtRT-PCR assay[2,3].

## RESULTS

Table 1. Detection of RV from various samples from striped skunks

| Sample type | Number positive samples | rtRT-PCR Positive | Percentage (%) |
|---|---|---|---|
| Brain | 32 | 32 | 100 |
| Oral Swab | 30 | 29 | 97 |
| Eye Swab | 29 | 27 | 93 |
| Combined swabs | 29 | 29 | 100 |
| Vibrissae | 29 | 22 | 76 |
| Corneal Impression | 26 | 15 | 58 |
| Salivary Gland | 32 | 10 | 31 |
| Ear | 28 | 9 | 32 |

Figure 1. Sensitivity and specificity of rtRT-PCR to detect RV in samples from naturally infected striped skunks in northern Colorado, USA, 2019. Black bars show 95% CIs for estimates.




- RV was not detected with rtRT-PCR in any sample types from seven skunks with negative rabies diagnosis by DFA.

## CONCLUSION

- Alternative sample types for RV rt-PCR diagnosis may be suitable in support of ERS
- Oral and eye swabs combined resulted in diagnostic sensitivity of 100%.

**Going forward:**

- Test larger sample sizes, targeting raccoons naturally infected with RV variant in comparison to brain diagnosis.
- Compare alternative rt-PCR probe sets.
- Evaluate alternative methods of swab sample collection and preservation.

References:
[1]Ma et al. (2024). Rabies surveillance in the United States during 2022. Journal of the American Veterinary Medical Association 262 (11), 1518–1525
[2]Wadhwa et al. (2017). A pan-Lyssavirus Taqman real-time RT-PCR assay for the detection of highly variable Rabies virus and other lyssaviruses. PLoS Neglected Tropical Diseases, 11(1), e0005258.
[3]Gilbert et al. (2024). Reemergence of a big brown bat Lyssavirus rabies variant in striped skunks in Flagstaff, Arizona, USA, 2021–2023. Vector-Borne and Zoonotic Diseases, 24(8), 552–562.

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