Rabid Kitten Sparks Multiagency Response to Stop Spread of Raccoon Rabies

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Good afternoon

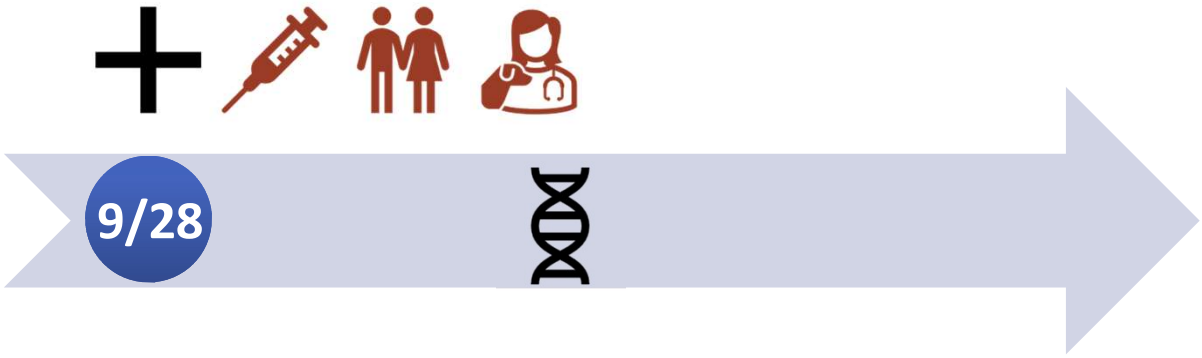
A 6-wk old stray kitten tests positive for rabies virus in Omaha, Nebraska.

On Thursday, September 28th, 2023, routine surveillance identified a 6-week-old stray kitten that had died of rabies in Omaha, Nebraska

This was the first domestic animal with rabies identified in Douglas County since 1981.

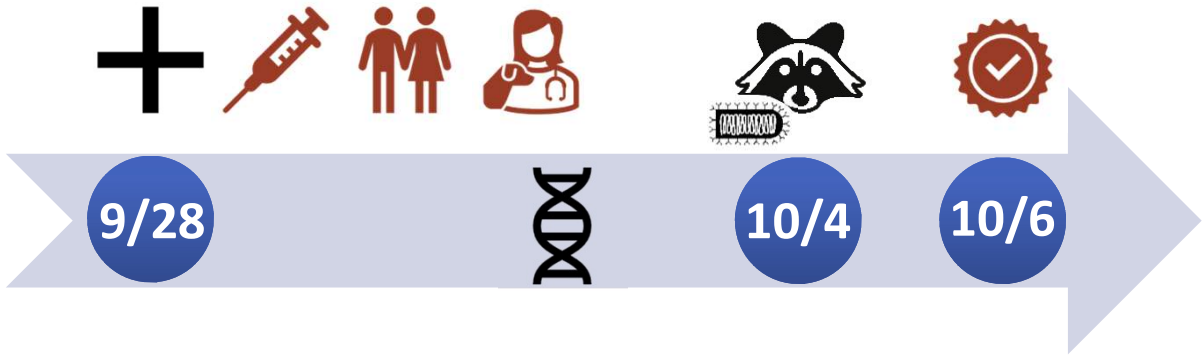
This was the first domestic animal with rabies identified in Douglas County since 1981.

10 persons exposed to the kitten began receiving rabies post-exposure prophylaxis (PEP).

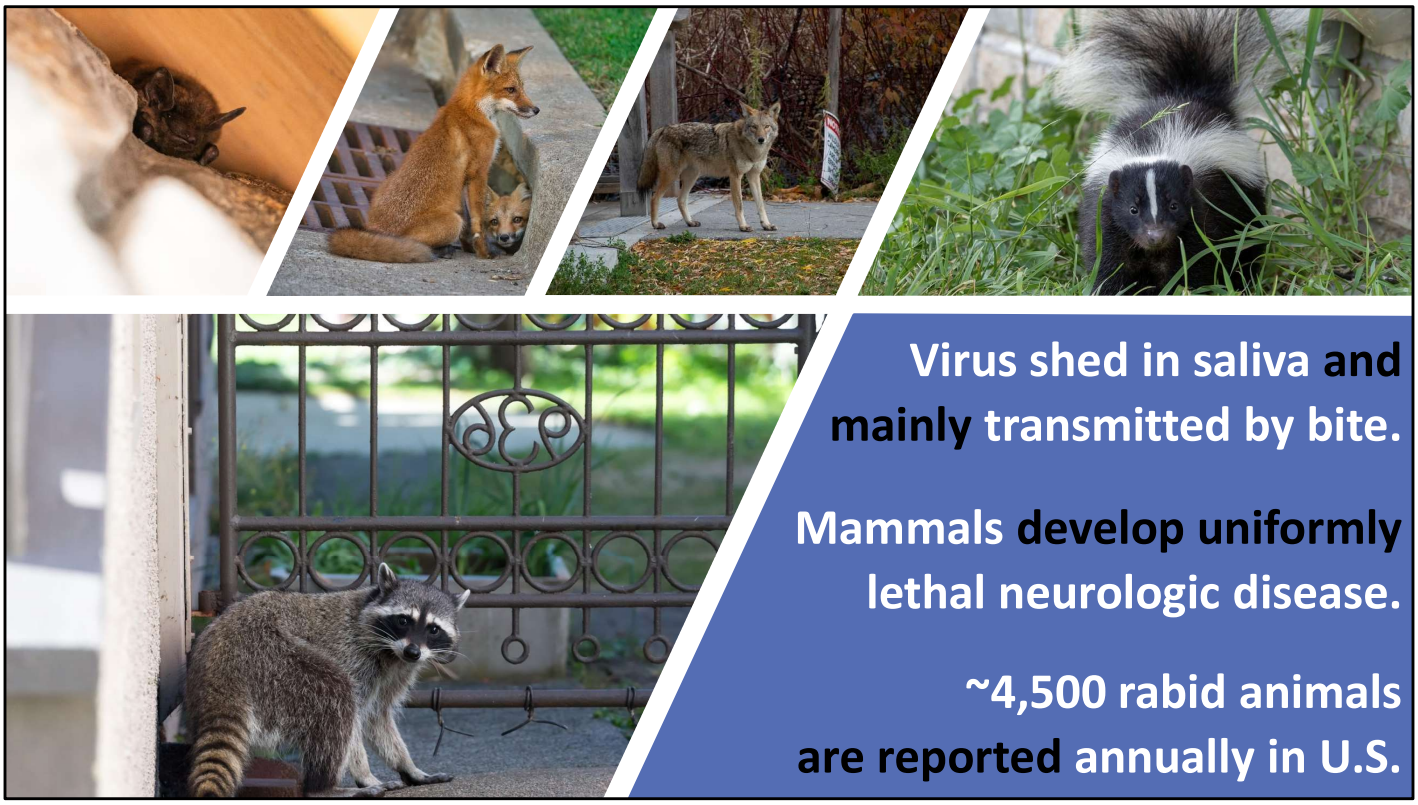


The local health department notified 10 persons exposed to the kitten, and all began receiving rabies post-exposure prophylaxis, or PEP. Given the rarity of a rabies positive domestic animal in Omaha, **(click)** non-routine sequencing of the virus was requested to determine the variant.

Sequencing of the kitten's virus was consistent with raccoon rabies virus variant.



On October 4th, Nebraska Veterinary Diagnostic Center reported the kitten's virus was consistent with raccoon rabies virus variant and **(click)** within 48 hours CDC confirmed this result.



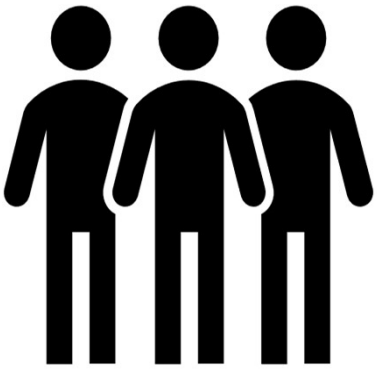
Rabies virus is shed in the saliva and is mainly transmitted by bites, **(click)**
Following an incubation period, mammals develop a uniformly lethal neurologic disease **(click)**
During the 2017-2022 surveillance years, an average of 4500 rabid animals have been reported each annually throughout the United States, and exposures to these animals result in tens of thousands of human rabies exposures.

(~60,000 people receive PEP per year in U.S. but is not always given due to proven rabies exposure)

Rabies is preventable through pre-exposure vaccination and post-exposure prophylaxis (PEP).

Thankfully, rabies is preventable through pre-exposure vaccination of those at high risk of rabies exposure and administration of post-exposure prophylaxis or PEP following a rabies exposure if given prior to symptom onset.

Rabies still kills 1-3 Americans annually.



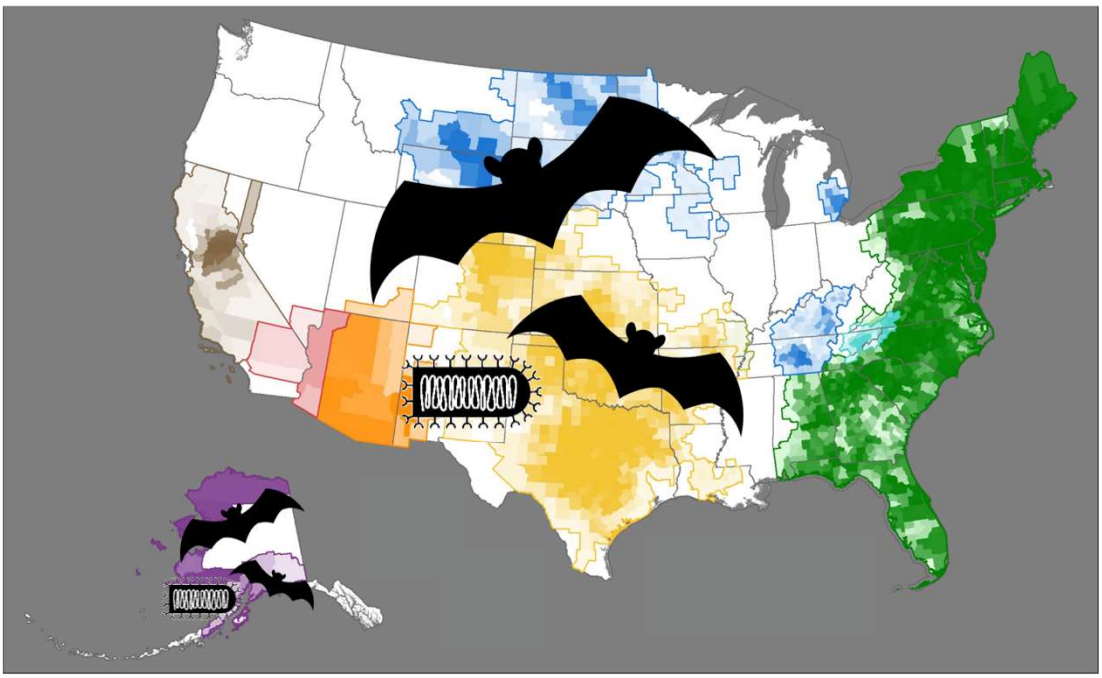
1-3 Deaths

70%

However, rabies still kills 1-3 Americans annually. **(click)** Among these, 70% are due to exposure to bat rabies virus variants

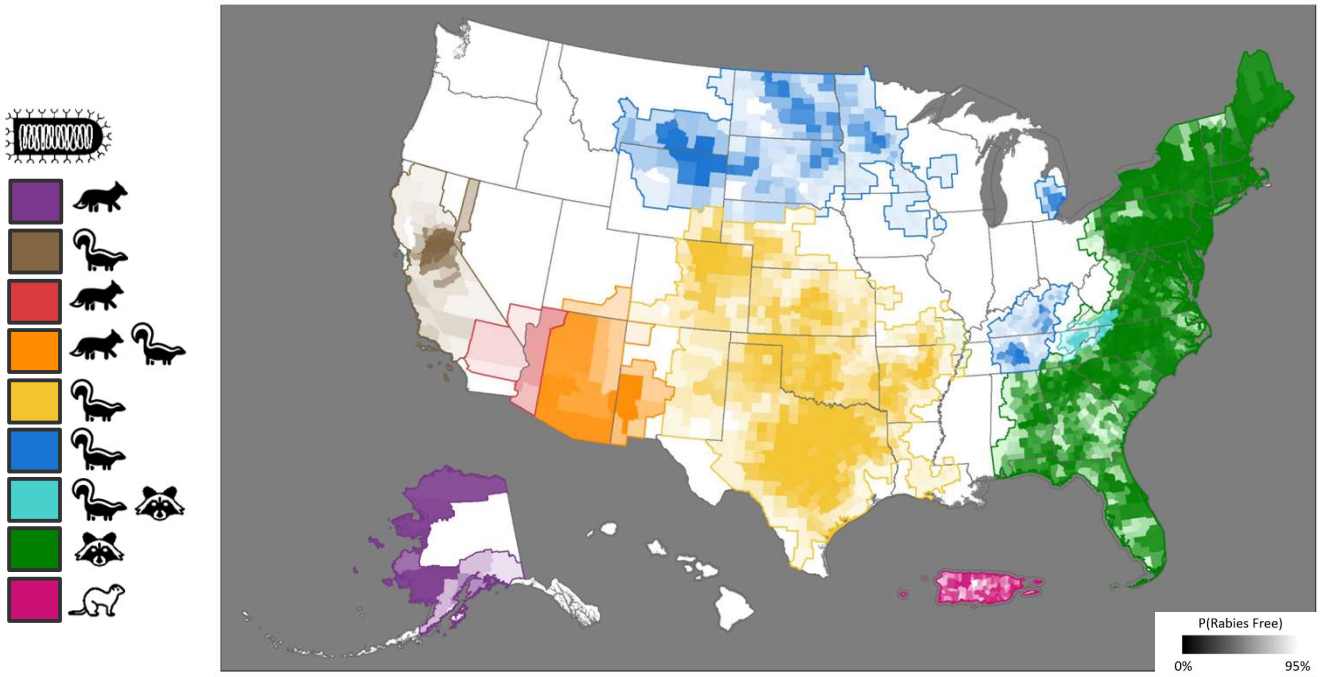
(Globally WHO estimates there are 59,000 human deaths annually due to rabies, however recent modeling suggests this could be as high as 70,000 due to underreporting; 99% are due to bites from rabid dogs; 40% of these fatalities occur in children under the age of 15; Southeast Asia and Africa have the largest burden of rabies.)

Bat rabies variants occur throughout the continental U.S.



Bat rabies virus variants are found in multiple bat species throughout the continental United States.

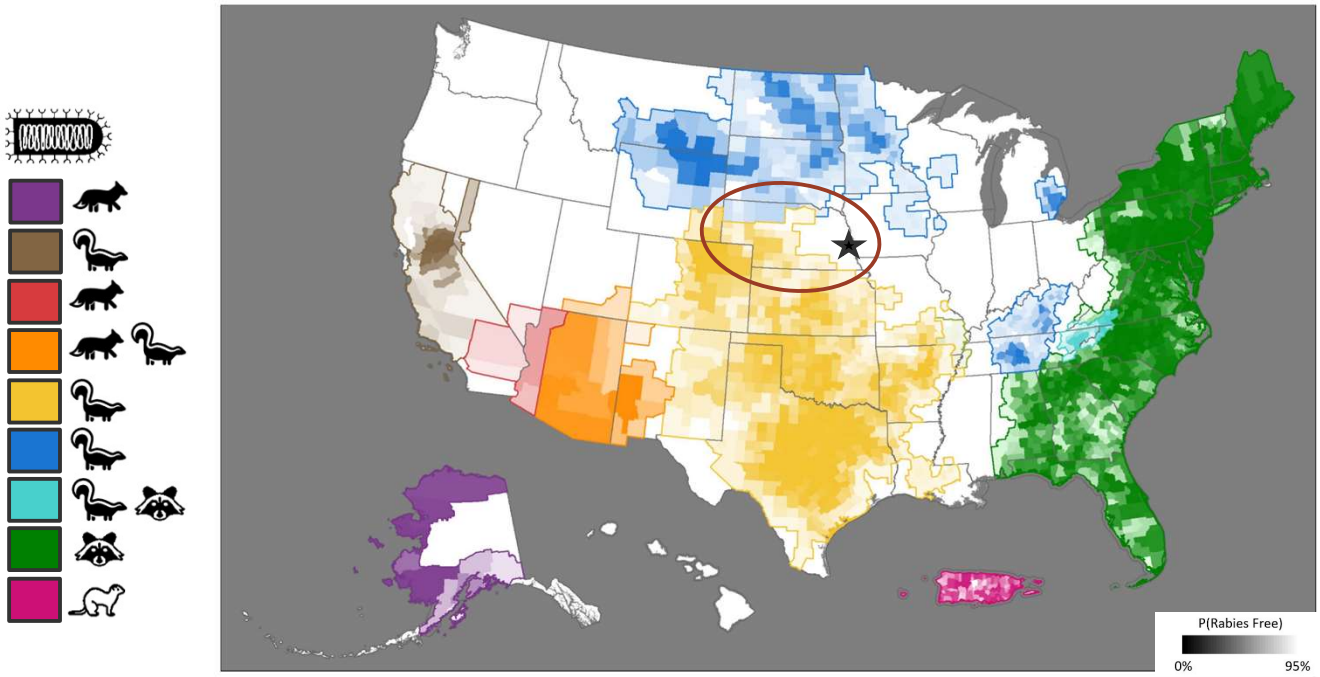
Terrestrial mammal rabies variants occur in distinct areas.



However, terrestrial mammal rabies variants occur in distinct geographic areas and are named for their location and primary reservoir species, although spillover into other species does occur.

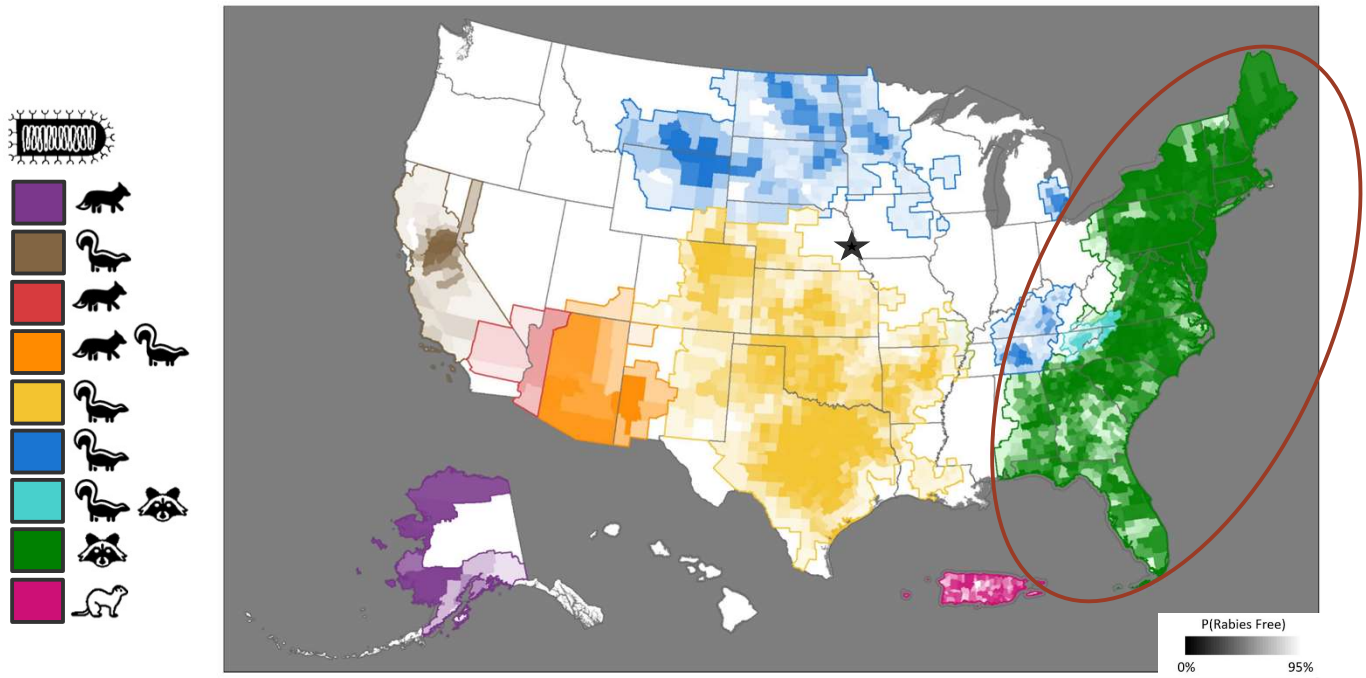
Parts of the U.S. that are free from terrestrial variants on this map are white, while colored areas indicate the variant, or variants, present as indicated in the key on the left, with darker shades indicating a higher incidence.

Nebraska is enzootic for skunk rabies virus variants.

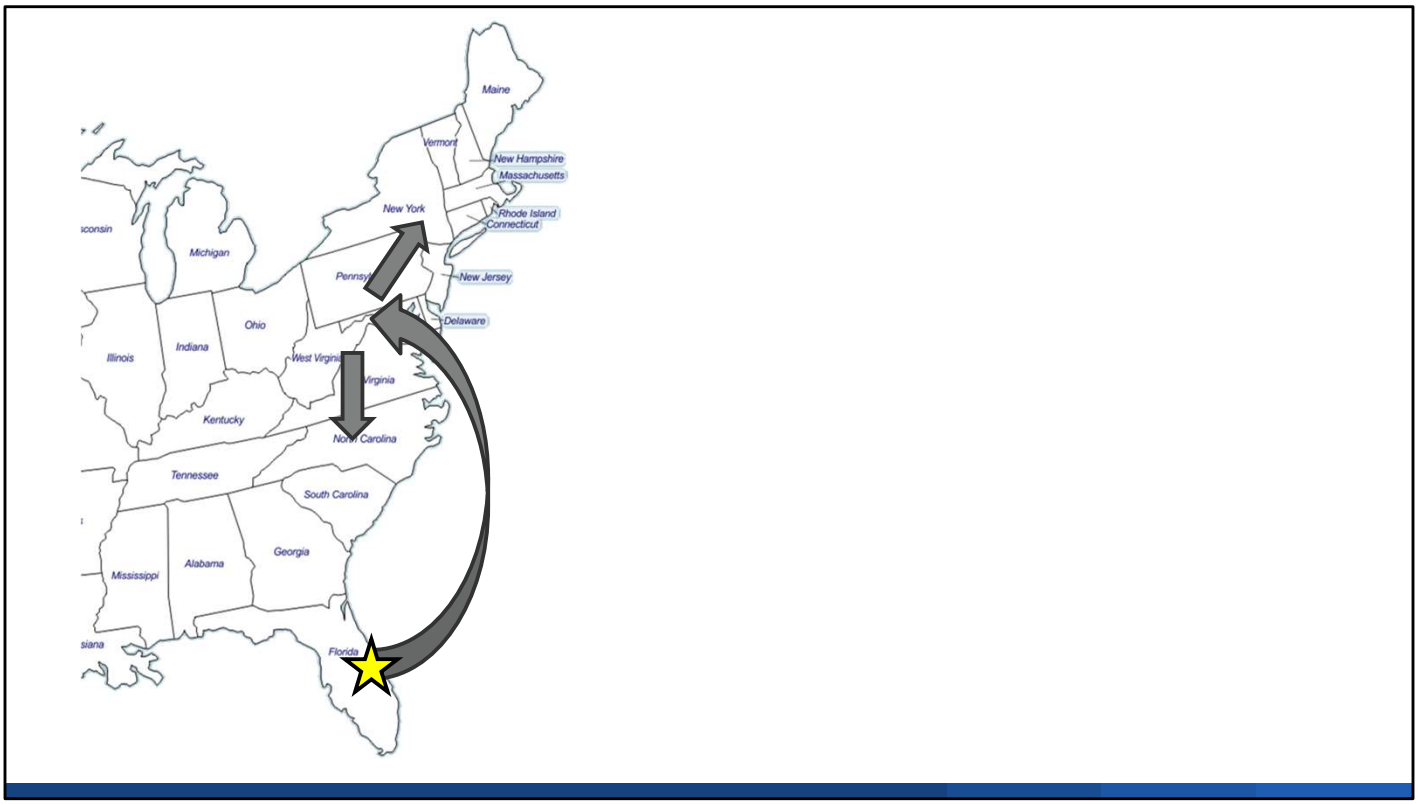


Nebraska is enzootic for two skunk rabies virus variants, the North Central skunk in dark blue and the South Central skunk in yellow. However, Omaha along the eastern border of Nebraska is currently free of terrestrial rabies variants.

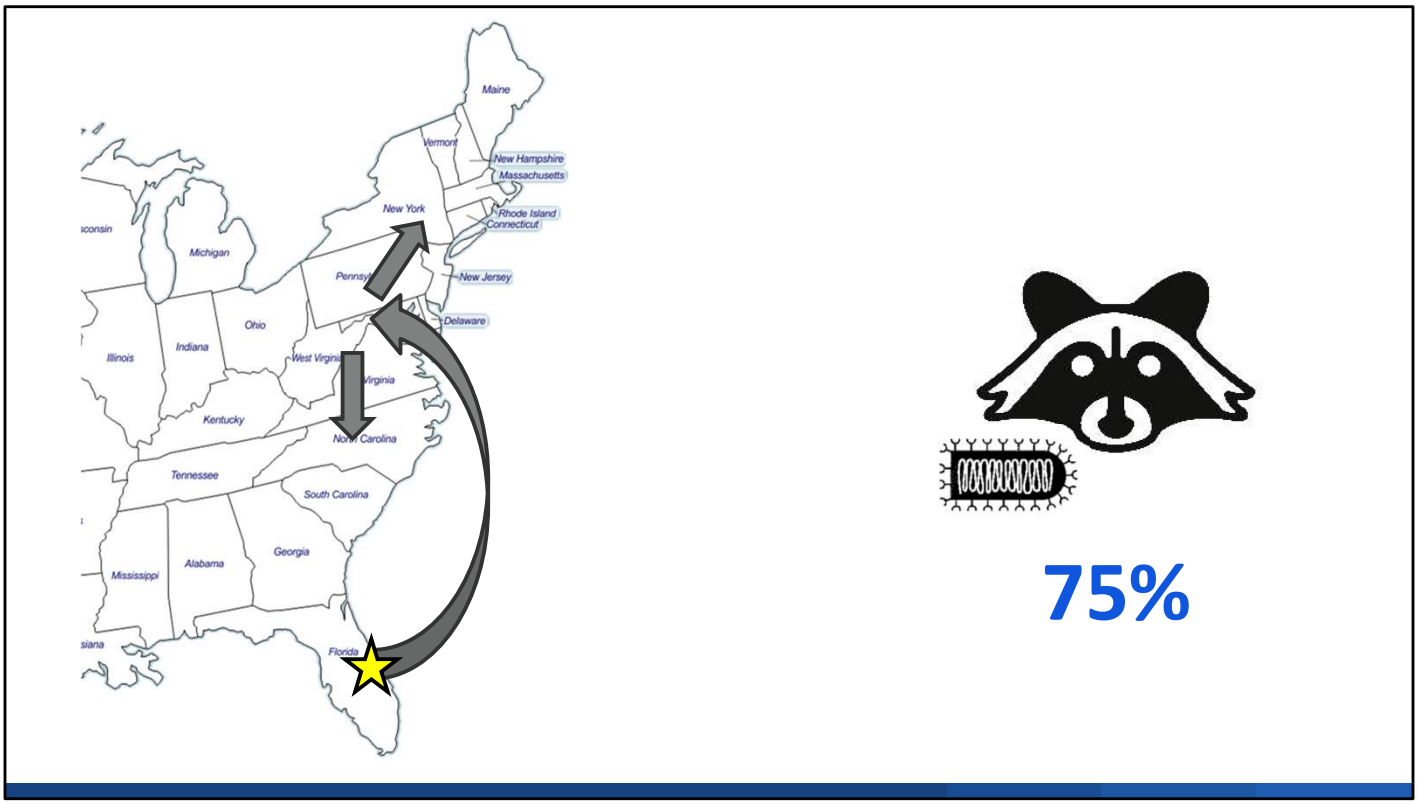
Raccoon rabies virus variant occurs in the Eastern U.S.



The kitten was infected with raccoon rabies virus variant which occurs along the entire Eastern U.S., with the closest enzootic area approximately 850 miles away from Omaha.



First recognized in Florida in the 1940s, raccoon variant was translocated to West Virginia in 1977 when raccoons were moved for hunting and quickly spread up and down the eastern seaboard.



Raccoon rabies now accounts for nearly 75% of all terrestrial animal rabies reported in the US

Raccoon variant enzootic areas report 600% higher human rabies exposures than those without a terrestrial variant…

Areas enzootic for raccoon variant report human exposures 600% higher than areas without a terrestrial rabies variant, in large part because raccoons naturally live in close proximity to humans and their pets.

…and have 70% higher PEP demand than areas enzootic for skunk variant, like Nebraska.

And these areas have 70% higher PEP demand than areas enzootic for skunk variant, like Nebraska.

What could happen if raccoon variant became established in Nebraska wildlife?

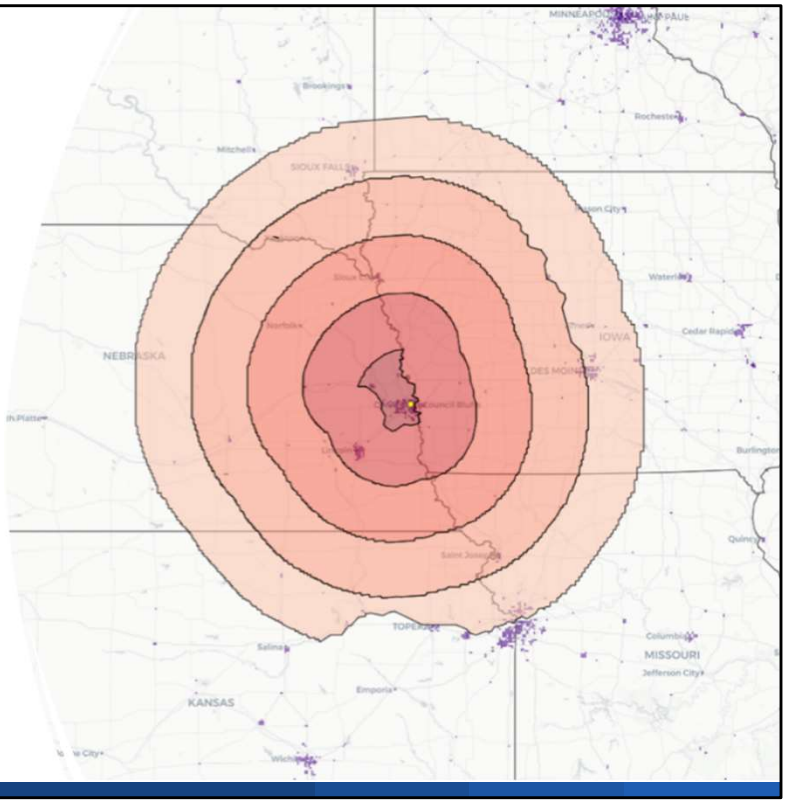
With these consequences in mind, we needed to know “What could happen if raccoon variant became established in Nebraska wildlife?”

60 km/year expansion

5 states in 5 years

2,000 – 12,000 human exposures

$17-105 million in PEP



Modeling predicted, raccoon variant could spread up to 60 km per year with minimal hindrance by local rivers. **(click)** Impacting 5 states neighboring Nebraska within 5 years. **(click)** resulting in up to 2 to 12,000 additional human exposures **(click)** and leading to another $17-105 million dollars in PEP and healthcare costs. (estimating a median cost of $8700 per person based on the findings from a 2018 Minnesota study and accounting for inflation)

(potentially impacted neighboring states: South Dakota, Minnesota, Iowa, Missouri, and Kansas)

Federal, state, and local partners conducted a One Health response.

Enhanced rabies surveillance

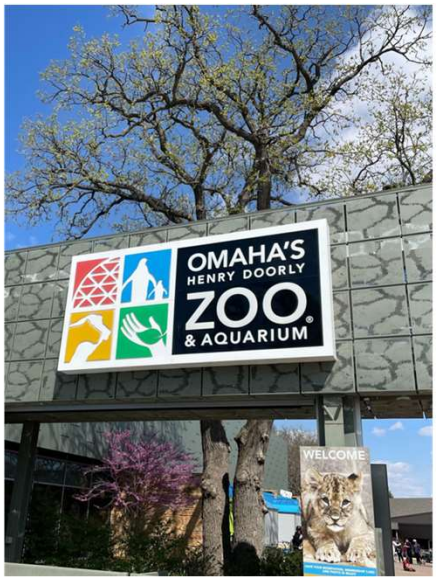
Mitigation through wildlife vaccination

To prevent this scenario, we worked quickly with our federal, state, and local partners to conduct a comprehensive One Health response which included many facets, but today I will focus on just two: **(CLICK),**

enhanced rabies surveillance **(CLICK)**

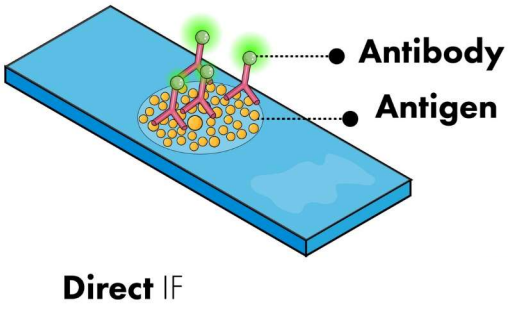
and mitigation through two methods of rabies vaccination in wildlife.

We utilized found dead and euthanized strange-acting wildlife to conduct enhanced rabies surveillance.

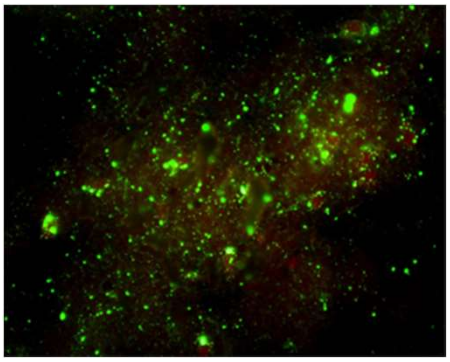


We utilized found dead and euthanized strange acting wildlife to conduct our enhanced rabies surveillance. which were already routinely collected by Animal Control. We additionally partnered with Nebraska Wildlife Rehab, the Henry Doorly Zoo, and USDA to collect any wildlife they euthanized for injury, nuisance control, or abnormal behavior.

The direct fluorescent antibody (DFA) test is the gold standard for diagnosing rabies.



Direct IF

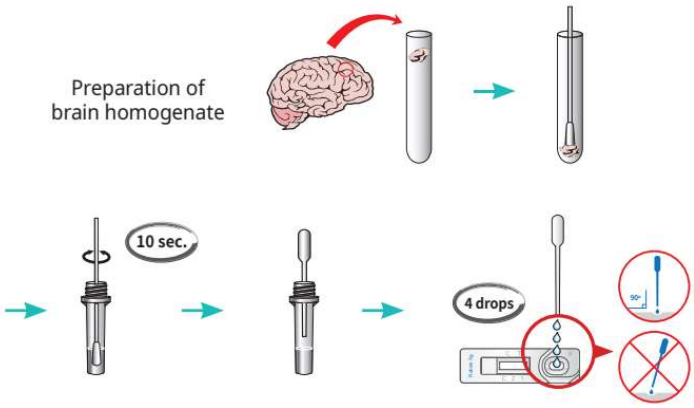


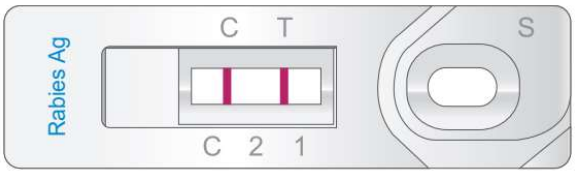
The direct fluorescent antibody or DFA test is the gold standard for diagnosing rabies and works by staining an impression of brain tissue with an antibody specific for rabies virus nucleoprotein that is fluorescently tagged. **(click)**

If rabies virus antigen is present the tagged antibody will bind and when viewed under a fluorescent microscope the brain impression will glow green. In addition to requiring specialized training and equipment, relatively fresh and intact brain tissue is necessary for DFA testing. Therefore, we needed a different testing modality to screen found dead wildlife, many of which were struck by vehicles and subjected to the elements for up to several days prior to collection.

(DFA costs $54.40 per test, takes 3 hour to perform from impression smear to microscopy, requires specialized equipment, and proficiency testing to be able to interpret the results)

Deploying a rabies lateral flow assay (LFA) to conduct enhanced surveillance in the U.S. for the first time.





Images from BioNote Anigen Rapid Rabies Ag Test kit insert

We instead chose to deploy a rabies lateral flow assay to conduct enhanced rabies surveillance for the first time in the United states. CDC has previously used this in low-resource settings internationally for enhanced surveillance for canine rabies virus variant. **(click)**

This LFA is a rapid test whereby brain homogenate is added to a swab, mixed with a diluent, then added to the test. **(click)**

If rabies virus antigens are present the test with show two red lines.

Why use an LFA?

- Rapid (10 min)
- Low cost
- Minimal training & equipment required
- Designed for field use
- Qualitative result

We chose to use the LFA test over DFA for several reasons including **(click)**

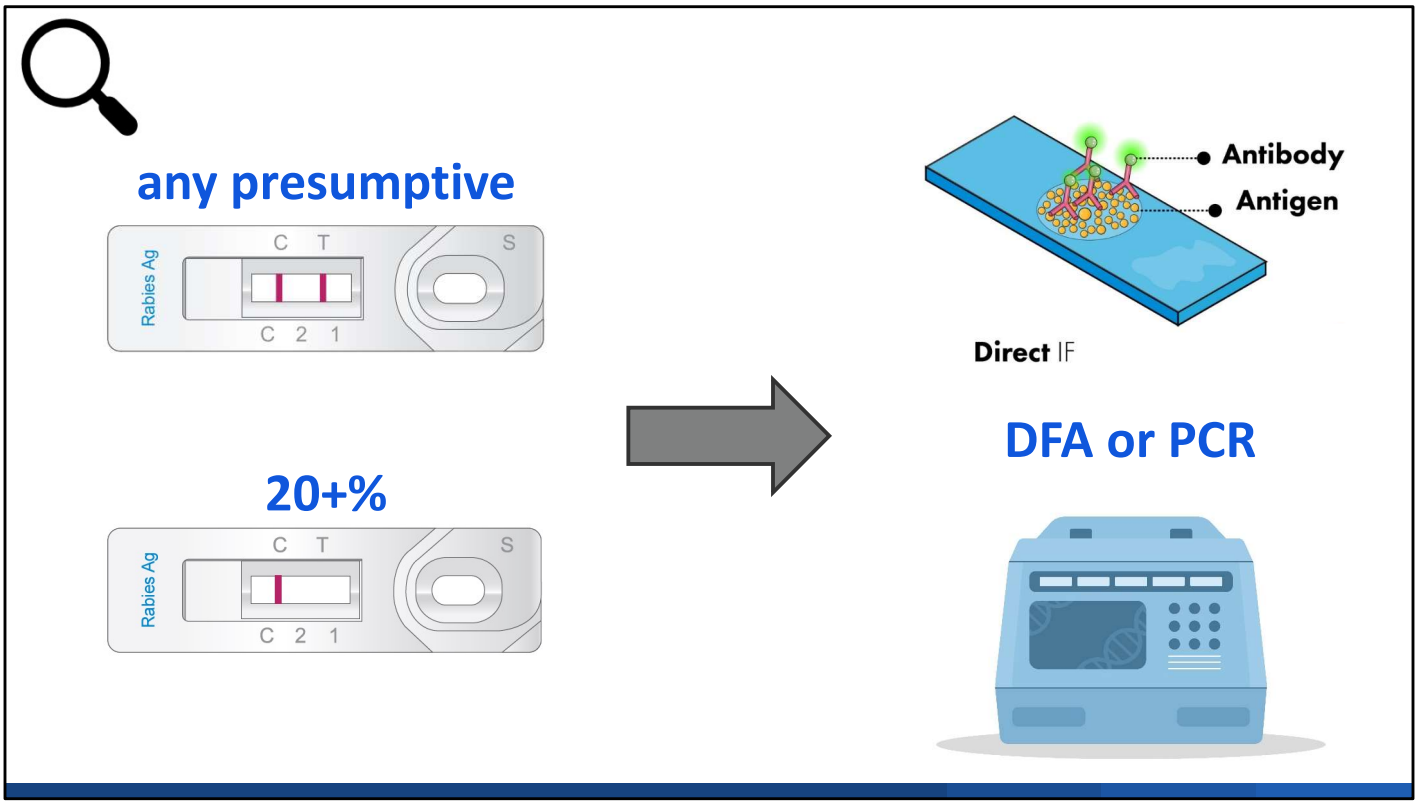
that it gives a rapid result within 10 minutes **(click),**

it's low cost at just $7-10 per test **(click),**

the test being very simple perform with only the supplies shown on the right required **(click)**

it does not need cold storage and can be used in the field as long as it's above 40 degrees F **(click)**

and it gives an easily interpretable positive or negative result.



Although this LFA has a 99% sensitivity and specificity using CDC's protocol, it is not yet a recognized rabies diagnostic in the United States. And so, we planned to test any presumptive positive and at least 20% of negative samples at CDC using DFA or polymerase chain reaction confirmatory tests.

Establishing a field laboratory for rabies screening.

Carcass storage

Biosafety

Waste disposal

Through the assistance of many partners, we were able to establish a field laboratory for LFA testing based out of the Nebraska DHHS emergency response trailer.

We acquired a lockable chest freezer to cold store carcasses **(click)**

For biosafety, we retrofit the trailer with a dead air box for testing, box fan for ventilation, and biohazard waste collection boxes **(click)**

And for biohazard waste and animal carcass disposal we utilized Nebraska Humane Society's large crematorium.

We used a modified dissection method to acquire brainstem for testing.

We used a modified dissection method on thawed carcasses to acquire brainstem at the joint between the first vertebrae and the skull. This technique was faster and safer than performing decapitations and opening the skull, which is done with DFA testing. Using this method, we were able to acquire upwards of 20 brainstem samples per day for testing.

We used an app to log specimen information in real-time.

CDC
Centers for Disease Control and Prevention

Tag the exact location on the map

Lat:	40.7946556758335
Long:	-96.68015499792…
Accuracy:	35.0m

VIEW MAP

Animal Symptoms:

- ☐ Hypersalivation
- ☐ Paralysis
- ☐ Lethargy
- ☐ Abnormal Aggression
- ☐ Abnormal Vocalization
- ☐ Diurnal activity of nocturnal species
- ☐ Other, specify:

Skull Condition:

- ○ Intact
- ○ Fractured, but recognizable
- ○ Severely fractured
- ○ Flattened
- ○ No structures recognizable

Brain Condition:

- ○ Acceptable quality: Optimum full cross section of brainstem
- ○ Good: Slight tissue decomposition but identifiable as brain
- ○ Poor: Discoloration, liquefaction, desiccation, or unrecognizable gross
- ○ Very Poor: Completely desiccated, maggots, skull fragments

Using the Worldwide Veterinary Services App, we logged information into the CDC's rabies field investigation database in real-time for each specimen including the species, location where found, any known symptoms, and sample condition.

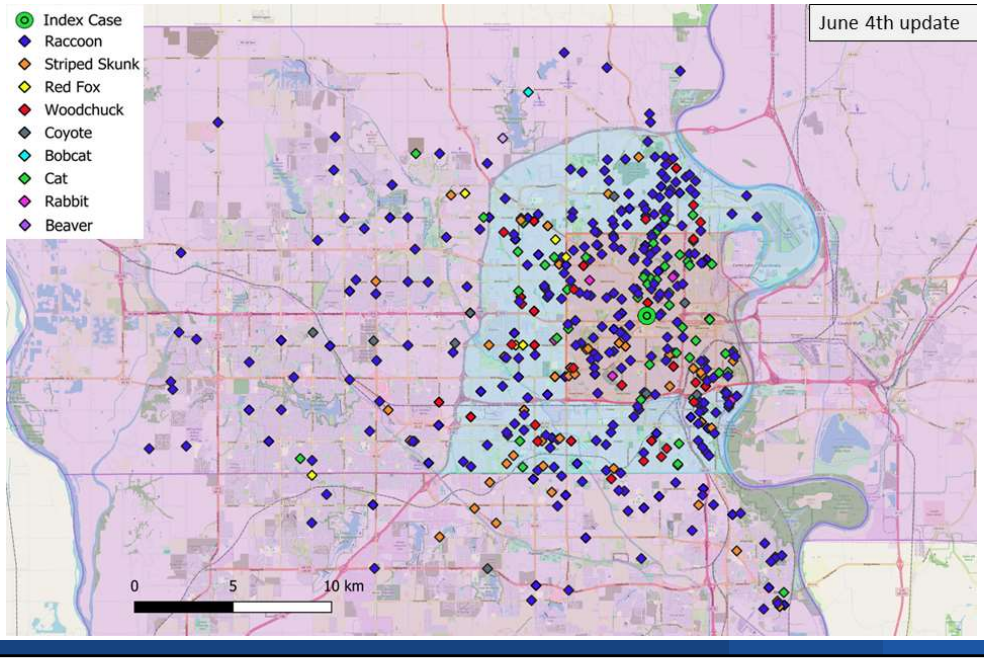
How many wildlife do we need to test through enhanced rabies surveillance?

459

But how many animals do we need to test through ERS to feel confident that raccoon variant has not emerged in Omaha wildlife? **(click)**

Our CDC rabies subject matter experts performed a complex sample size calculation and determined we need to test 459 high risk wildlife within an approximately 10 km area surrounding the index kitten to have a 99% probability freedom of rabies. Skunks, coyotes, woodchucks, and foxes were also are eligible for inclusion as they are common spillover species for raccoon rabies virus variant.

Enhanced and routine surveillance of 626 feral cats and wildlife has found no additional cases of rabies.



As of July 24, 2024, enhanced and routine rabies surveillance of 626 wildlife and feral cats, the majority of which have been raccoons, from the greater Omaha area has found no additional cases of rabies.

Using USDA's ERS point value system, we've surpassed our surveillance goal.

As of 7/30/2024:
332 eligible animals tested

Surveillance Method	Estimated Points
Strange acting	14
Found dead	20
Roadkill	4
Surveillance trapped	1
NWCO/Other	1
Unknown	12

Goal: 459 x 4 = **1,836 points**

Accumulated points
All species weighted = **2,909 points**
Only raccoons weighted = **2,360 points**

Davis et al., 2021 *Viruses*

As of July 30th we've tested 3032 animals eligible for inclusion into this sample size through enhanced rabies surveillance. (click)

However, USDA has validated a point system for raccoons based on the method of carcass collection to enhance their raccoon variant ERS efforts.

Assuming all 459 animals in our calculated sample size were roadkill, our ERS goal would be 1836 points.

Fortunately, we've tested many strange acting and found dead carcasses, which are much more valuable diagnostic samples, which has allowed us to surpass our surveillance goal whether we apply the weights to all the high-risk spillover species or just raccoons.

The absence of additional rabies detection suggests a recent translocation of raccoon variant to Omaha.

The absence of additional rabies detection suggests a recent translocation of raccoon variant to Omaha, with no establishment into Nebraska wildlife

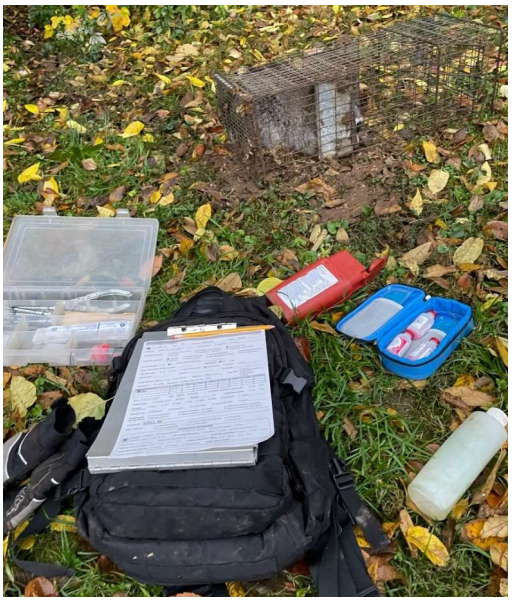
Federal, state, and local partners conducted a One Health response.

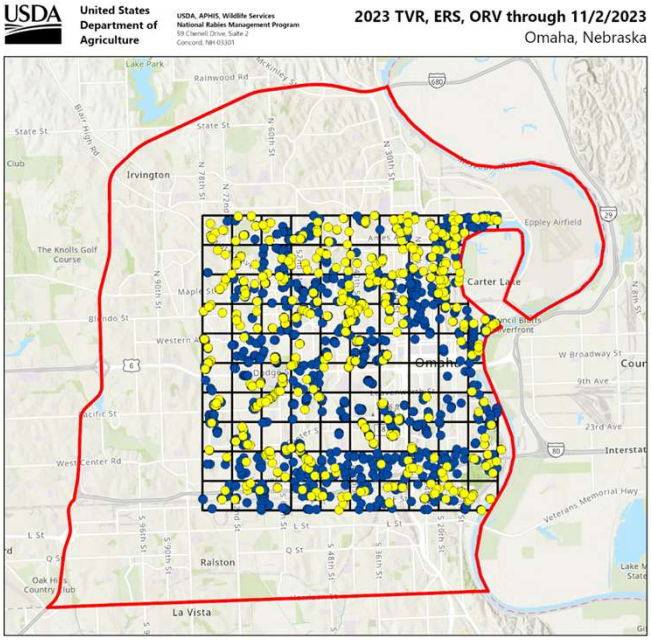
Enhanced rabies surveillance

Mitigation through wildlife vaccination

Now I'll briefly highlight USDA's mitigation efforts through wildlife vaccination.

USDA conducted trap-vaccination-release of 799 wildlife within 5 km of index kitten.

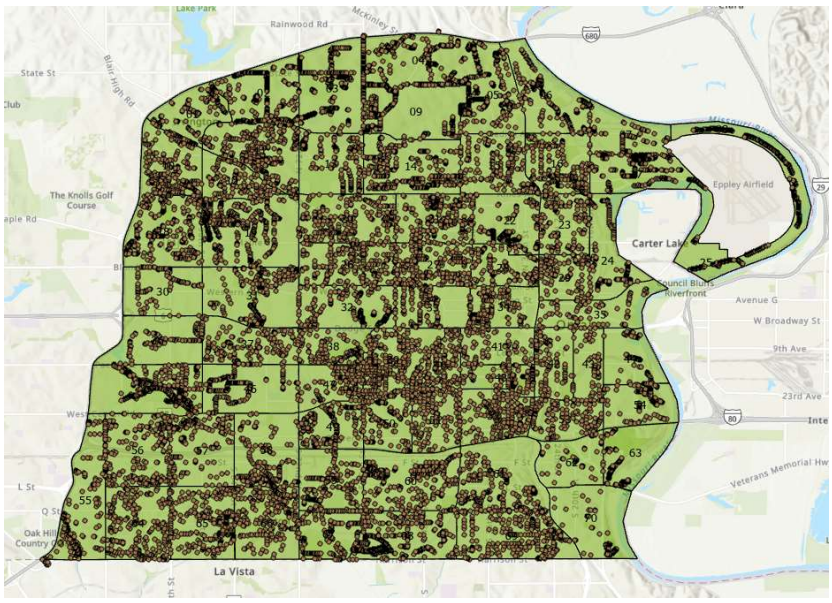




Twelve USDA wildlife biologists conducted trap-vaccinate-release of nearly 800 wildlife within 5 km of the index kitten. 95% of which were raccoons. This map depicts the GPS location of set traps with blue dots and vaccinated raccoons with yellow dots.

(96 square kilometers; nearly 6700 trap nights, team also vaccinated 42 skunks, 4 feral cats, and a red fox)

USDA distributed 18,000 oral rabies vaccine baits within 10 km of index kitten.



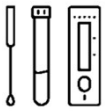
USDA hand- distributed 18,000 oral rabies vaccine baits within 10 km of the index kitten along suitable raccoon and skunk habit. These baits are small ketchup-packet sized sachets covered in a fishmeal bait to attract wildlife, which confers immunity against rabies virus when ingested. This map depicts the GPS location of bait placement show by brown dots.

(162 square kilometers; aim: 150 baits/km2 in targeted raccoon and skunk habitat)

Takeaways from this One Health response:

Animal translocation can spread rabies variants.

Increased variant typing is necessary to detect and respond translocation events.



LFA tests are ideal for enhanced rabies surveillance.

Maintaining high levels of rabies vaccination among domestic animals prevents spillover from wildlife.

While we don't know exactly how this case of raccoon variant arrived in Nebraska, animal translocation can spread rabies variants to new geographic locations **(click)**

Increased variant typing of rabid animals is necessary to detect and respond to translocation events like this one **(click)**

LFA tests are a novel modality to rapidly screen for rabies virus making them ideal for enhanced rabies surveillance **(click)**

And maintaining high levels of rabies vaccination among domestic animals is critical to preventing spillover from wildlife species.

I would like to thank and acknowledge all of our One Health partners who contributed to this response

Dr. AJ Beron
EIS ‘22

Dr. Sergio Rodriguez
LLS ‘22

Dr. Victoria Shelus
EIS ‘22

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EIS ‘22

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EIS ‘22

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Kathleen Nelson
Amy Hess

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And especially the other members of the Epi-Aid team, Drs. AJ Beron, Sergio Rodriguez, Victoria Shelus, Emma Price, and Ann Carpenter along with the CDC Poxvirus and Rabies Branch and USDA APHIS Wildlife Services.