

Enhanced Rabies Surveillance in Alaskan Wildlife: Detection of New Host Range and Risk to Trappers



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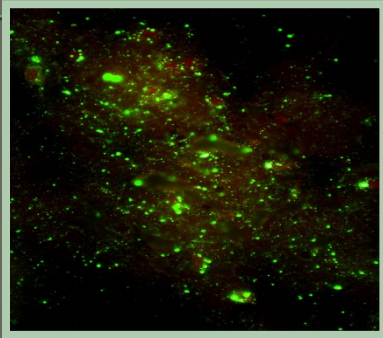


David R. Sinnott

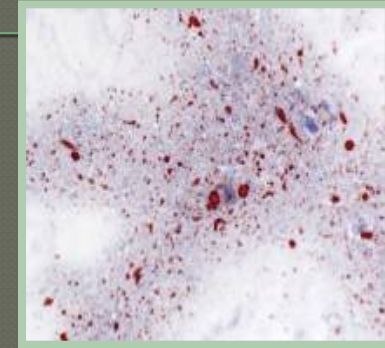
U.S. Department of Agriculture, Wildlife Services
Palmer, Alaska

Introduction

Direct fluorescent antibody (DFA) test
Indirect fluorescent antibody test (mAb)



Direct rapid immunohistochemistry test (dRIT)



Antigen Detection

Laboratory Techniques in Rabies

Virus Isolation



Mouse Inoculation

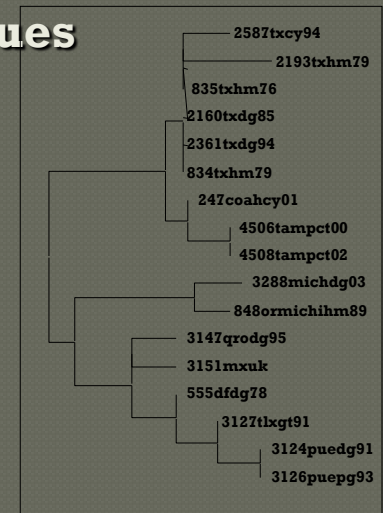


Cell Culture



Molecular Techniques

**RT-PCR
Sequencing**



Slide credit M. Niezgoda



Introduction: Advantages of dRIT

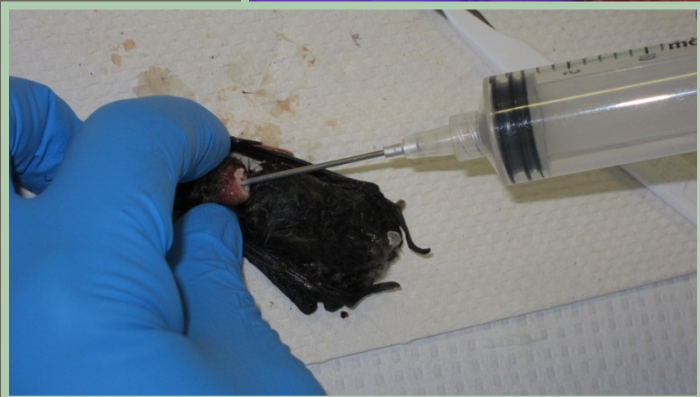
- Offers real-time diagnostic results in less than 1 hr.
- All work can be done at bench-top at ambient temperature.
- Requires no specialized equipment such as a fluorescent microscope or incubator.
- As a colormetric test the only equipment required is a standard light microscope.
- The dRIT permits for rabies diagnosis under field conditions without the need for electricity.

Slide credit M. Niezgoda

Lembo, T. et al. 2006. Evaluation of a Direct, Rapid Immunohistochemical Test for Rabies Diagnosis. *Emerging Infectious Diseases*. 12(2): 310-313.

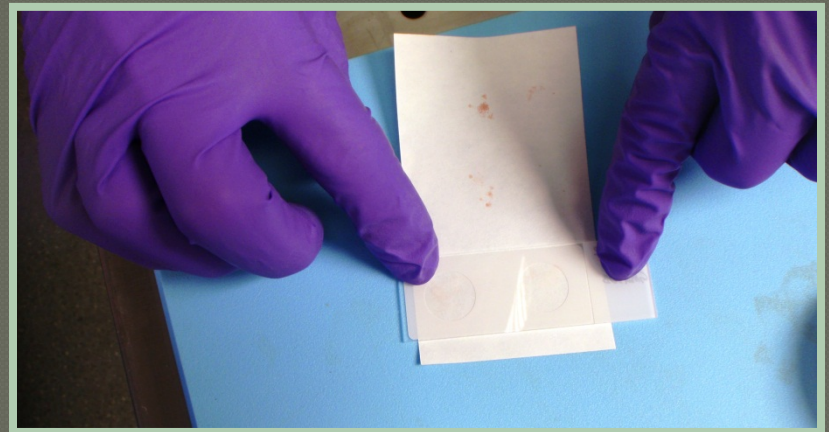
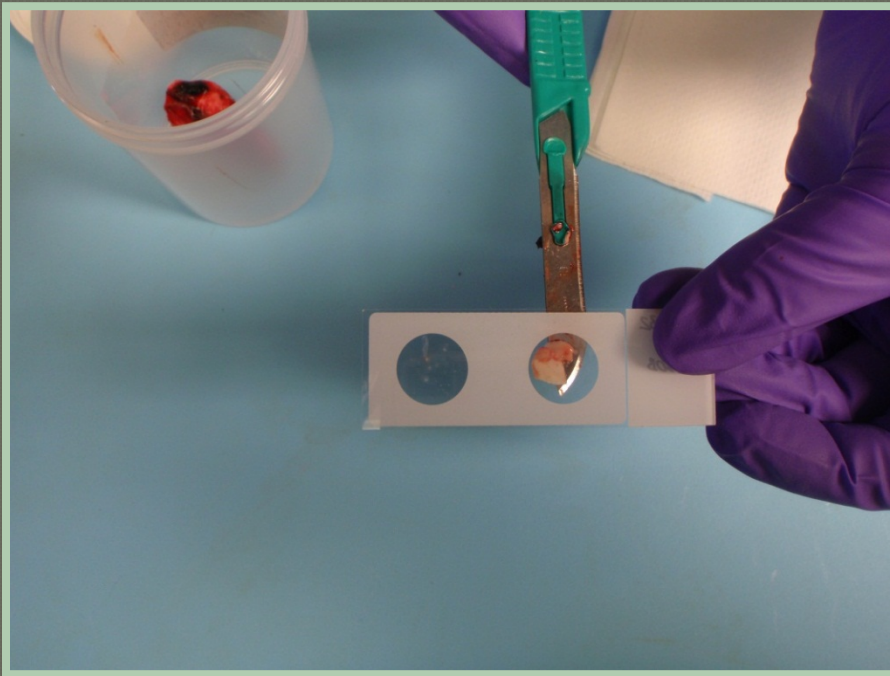
Methods: Sample Collection Technique

- Collect brainstem through foramen magnum



Methods: Slide Preparation

- Make touch impression onto slide



- Blot & air dry
- Can be stored at -40C

Methods: Staining Procedure

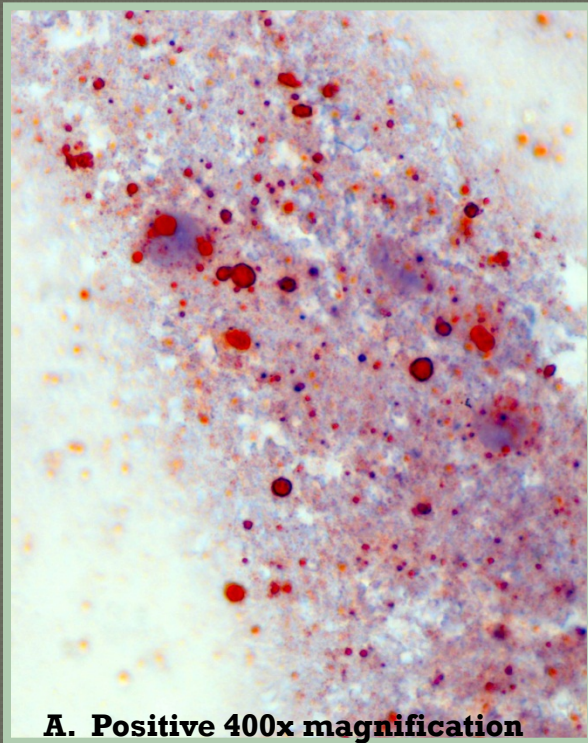
- 12-step staining procedure using mouse anti-rabies biotinylated monoclonal antibodies



- ~1 hour to process 25 slides

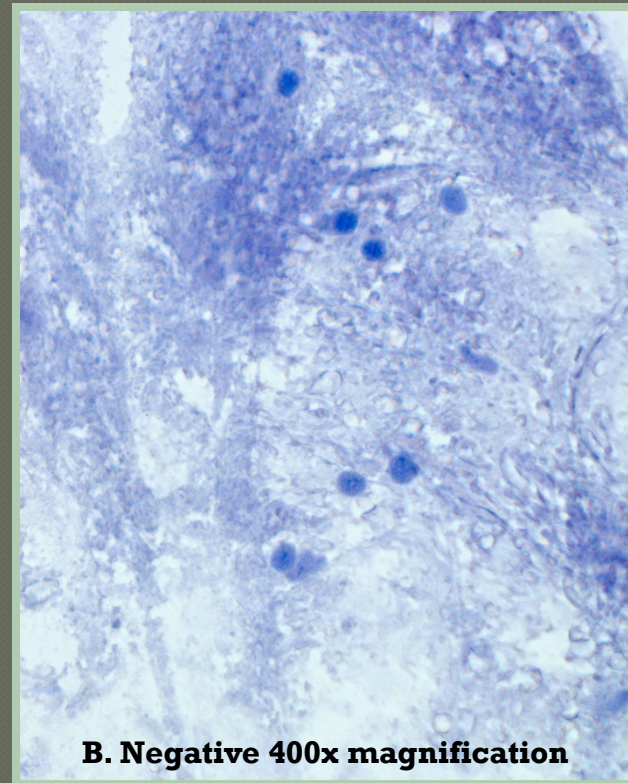


Light Microscopy



A. Positive 400x magnification

Positive: bright red inclusions (rabies virus nucleoprotein) of varying shape & size distributed throughout slide

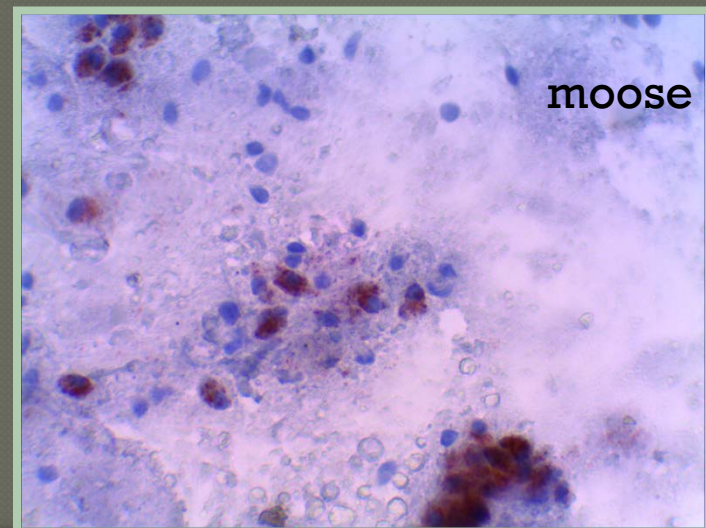
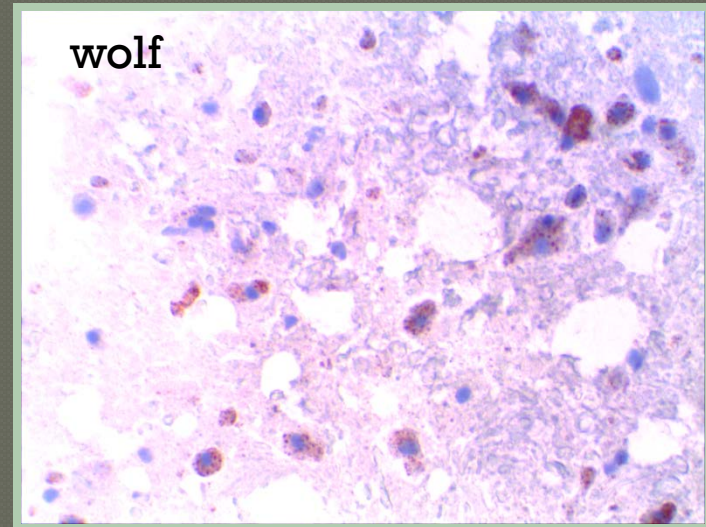


B. Negative 400x magnification

Negative: absence of bright red inclusions

Methods

- **Indeterminant result:**
Bright red inclusions that are intracellular, often only present in 1-2 fields of view/slide.
- All positive or indeterminate samples plus 10% of each tester's negatives are submitted to the CDC for confirmation by DFA, RT-PCR and for typing.



Results

- Currently, USDA/WS and ADF&G are the only wildlife management agencies certified to perform dRIT
- 1566 brainstem specimens from 21 species tested in Alaska via dRIT from 2011 to November 2013
 - 65% → trapper/hunter harvested, predator control
 - 18% → animals killed by vehicular trauma
 - 17% → from necropsy cases, found dead, or dispatched due to abnormal behavior.

Results

- Rabies positive (+) by dRIT, confirmed by DFA in 4 species:
 - Arctic Fox
 - Red Fox
 - Wolf
 - Wolverine- first case in North America
- Indeterminant (I) results $n=34$
 - 55% of bat and 7.8% of other samples tested classified as (I)
 - All but one (I) tested by DFA were rabies (-)

dRIT validation compared to DFA

- Sensitivity=ability of a test to correctly classify an individual as *diseased*. 100% indicates no false negatives
- Specificity =ability of a test to correctly identify an individual as *disease-free*. 100% indicates no false positives

Species	dRIT Sensitivity*	dRIT Specificity
Arctic Fox (n=205)	88.9% (1 indeterminant dFA +)	100%
Red Fox (n=508)	100%	99.2% (4 FP)
Wolf (n=114)	100%	97.3% (3 FP)
Wolverine (n=49)	100%	97.9% (1 FP)
Little Brown Bat (n=18)	<i>na (no true positives)</i>	72.2% (5 FP)

*all dRIT positive and indeterminants validated by DFA however results of DFA on the 10% negatives not yet received.

dRIT Predictive Value

- Positive Predictive Value= the percentage with a *positive* test who actually *have* the disease
- Negative Predictive Value=the percentage with a *negative* test that *do not* have the disease

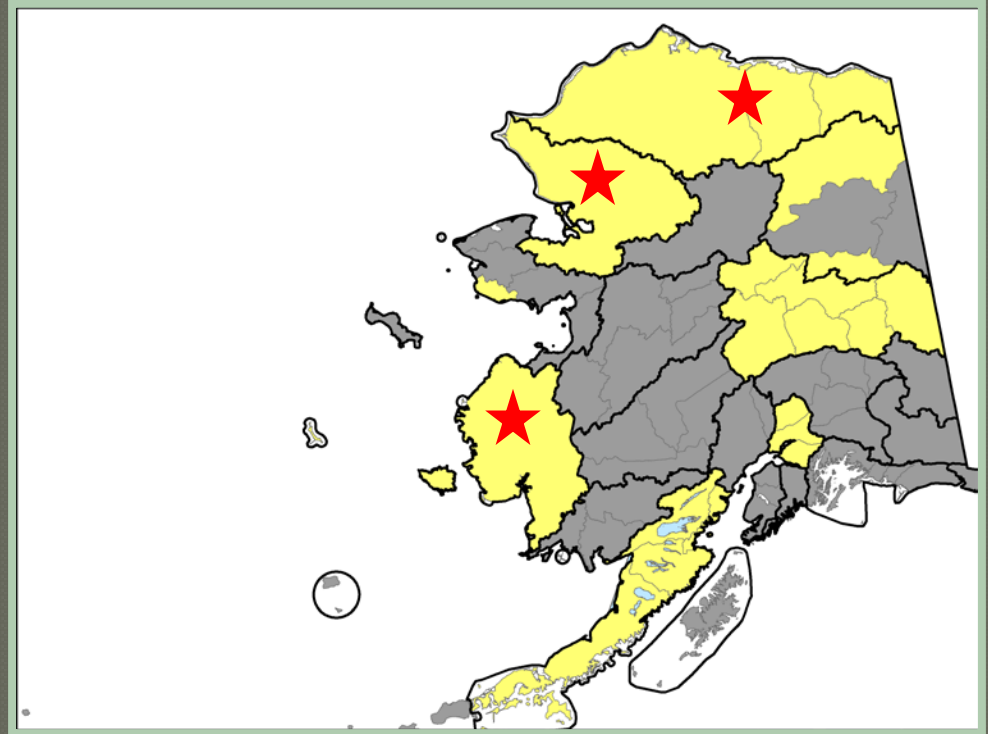
Species	PPV	NPV
Arctic Fox	100%	99.5%*
Red Fox	71.4%	100%
Wolf	25%	100%
Wolverine (n=49)	50%	100%
Little Brown Bat (n=16)	<i>na</i>	100%

*an indeterminant test result, not actually dRIT negative

Red Fox



■ Regions of Alaska where rabies is considered enzootic (always present at a certain level) among foxes



Yellow = GMUs represented in testing
★ = ≥ 1 rabies positive case in GMU

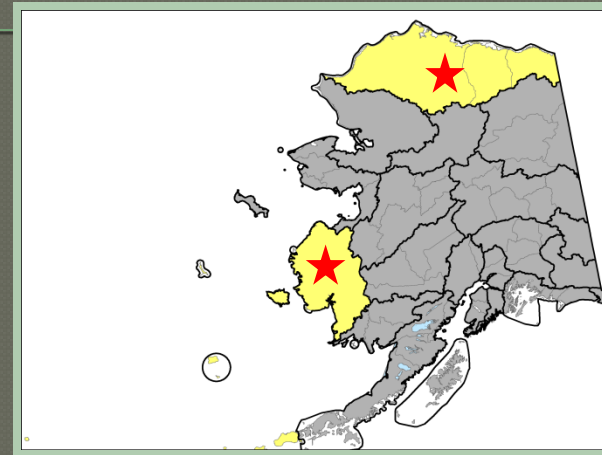
Red Fox, continued

Location	# Tested	# dRIT Positive	% Rabies Positive	# Known Trapper Caught	% Trapped & Rabies Positive
ALL (thru Jul 2013)	513	18, 4*FP	3.5%	473	1.3%
Alaska Peninsula	2	0	0%	0	N/A
Southcentral Anchorage / Palmer	57	0	0%	56	0%
Southwest /Bethel	344	5, 2 FP*	1.5%	344	1.5%
Interior / Fairbanks	22	1 FP*	0%	10	0%
Northwest Alaska	71	7, 1 FP*	20.5%	61	0%
North Slope	14	6	42.8%	0	N/A

“% Positive” does NOT include false-positives

*** = false-positive , 18 indeterminates (3.5% of total)**

Arctic Fox



Location	# Tested	# dRIT Positive	% Rabies Positive	% Trapped & Rabies Positive
ALL (thru Jul 2013)	204	9	4.9%	1.5%
Pribilof Islands	65	0	0%	N/A
Southwest	28	1	3.6%	0%
North Slope	111	8	8.1%	2.7%

- 2 indeterminates (0.9% of total) of which 1 was rabies positive

Rabies Prevalence: Foxes trapped in the enzootic region

Epizootic Year



Non-Epizootic Year



Red Fox

Winter 2009-2010
3.0% (n=135)



Winter 2010-2011
1.0% (n=209)

Arctic Fox

Spring/Summer 2013
5.0% (n=20)



Spring/Summer 2012
2.4% (n=82)

Rabies Prevalence: Other than trapped foxes

Cause of Death:

Vehicular trauma



Red Fox

14.3% (n=7)

Arctic Fox

42.9% (n=7)

Cause of Death:

Killed due to behavior



Red Fox

50.0% (n=16)

Arctic Fox

75.0% (n=4)

* One (+) red fox is counted in both columns: it attacked someone, was fought off, then ran away and was hit by a truck.



Wolves

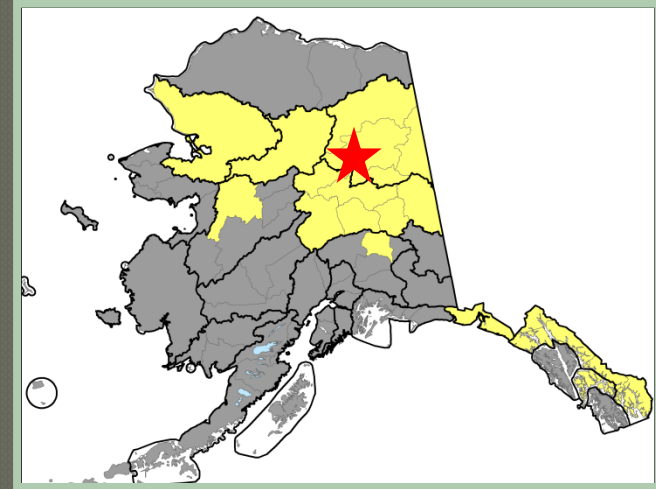
*false positive

9 indeterminates
(7.4%)

Location	# Tested	% dRIT Positive	% Positive & Trapped
ALL (thru July 2013)	121	1	0.9%
Central Interior	79	2*	N/A
Northern Interior	36	1	2.8%
Southcentral	1	0	N/A
Northwest	1	0	N/A
Southeast	4	1*	N/A

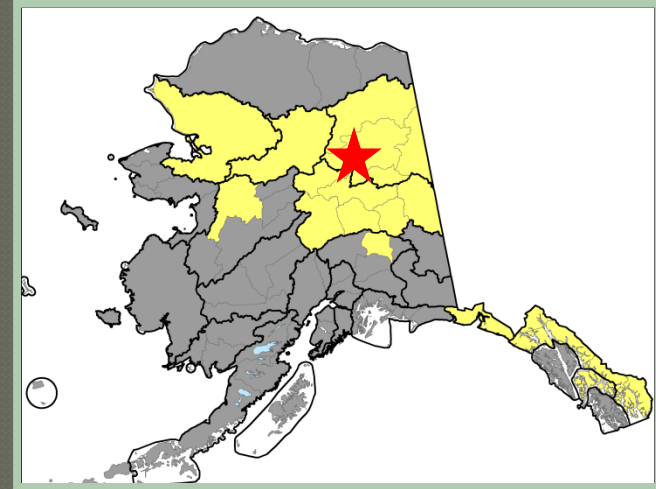
Range expansion or enhanced surveillance?

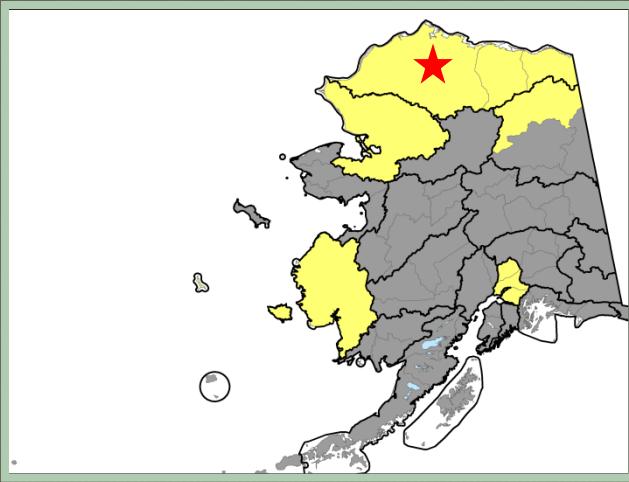
- Early Spring 2013, wolf skinner cut
 - Redirected to public health lab:
Rabies (+) by DFA.
 - Carcass already discarded in Southcentral
- A second wolf trapped ~16km away was dRIT (+) (confirmed by DFA).
 - Carcass fed to dog team in Fairbanks
- Unable to obtain heads of other wolves killed DLP in the area with suspect abnormal behavior due to logistical constraints



Range expansion or enhanced surveillance?

- ◉ Arctic fox variant
 - First cases of rabies (other than transport of HBV fox) documented in the Interior since 1945-47
 - A potential range expansion?
 - Failure of potential exposure surveillance only to detect extent of enzootic range?
 - Result of increased (exposed) NS predators responding to unusual caribou overwintering behavior?





Wolverine



First-ever documented case of rabies in a wolverine in North America (2012).

Location	# Tested	# dRIT Positive	% Rabies Positive
ALL	58	1	1.7%
Anchorage	1	<i>1*</i>	0%
Bethel	46	0	0%
Interior	6	0	0%
Kotzebue	3	0	0%
North Slope	2	1	50.0%

- ** = 1 false positive*
- 2 indeterminates (3.4% of total)
- 45 of 58 are trapped animals, 0% positive

Little Brown Bats

Myotis lucifugus

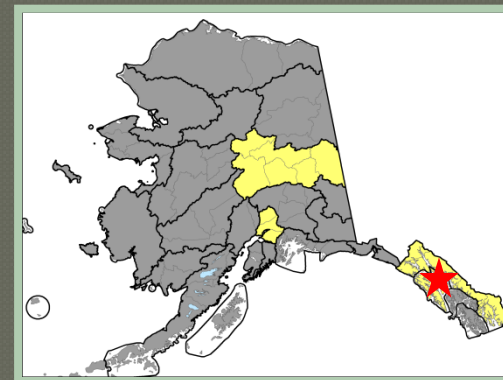


Location	# Tested	# dRIT Positive	% dRIT Positive
ALL	31	0	0%
Interior / Fairbanks	2	1 FP*	50%
Southcentral/Anchorage/ Palmer	19	0	0%
Southeast	10	4 FP*	40%

- High incidence of indeterminate results (n=17) and false-positives* (n=5).
- CDC reports higher sensitivity and specificity for dRIT in bats.
- 4 FP and 1 dRIT (-) verified by RT-PCR



Only DFA rabies positive *Myotis* spp. from Alaska 1993, 2006





Other species: all dRIT negative

Species	# Tested	# (I)
Moose	516	22
Caribou	30	0
Dall's Sheep	2	0
Muskox	2	0
Black Bear	5	1*
Brown Bear	26	7*
Polar Bear	1	0
Coyote	3	1



Species	# Tested	# (I)
Beaver	1	0
Ermine	2	0
Lynx	9	0
Muskrat	1	0
Pine Marten	18	0
Porcupine	1	0
River Otter	2	0
Snowshoe Hare	1	0
SBT deer	1	0

Summary of dRIT in this study

- 1) Identified an epizootic not observed by public health surveillance of exposure risk cases
- 2) Documented a 1.0 – 5.0% prevalence rate among 'normal' trapped foxes
- 3) Documented 42.9% of HBV foxes rabies (+)

Summary, continued

- 4) Identified rabies in a previously undocumented species (wolverine)
- 5) Facilitated the detection the most easterly case of rabies, south of the Brooks range in 66 years

Summary, continued

- 4) Test specificity and sensitivity are very high for foxes, wolves, wolverines but not bats
- 5) Negative predictive values were equivalent to DFA for all species tested
- 6) Positive predictive value is high for arctic fox, very good for red fox, wolverines but less than expected for wolves
 - Acceptable in a surveillance (e.g. no human exposure) context

Conclusions

- dRIT demonstrated efficacy for a wildlife agency to use for early detection of a rabies outbreak or changes in range distribution or prevalence utilizing HBV and hunter-killed (only available for this non-destructive sampling)
- Surveillance by these methods facilitates:
 - Early communication of rabies risk to the public, especially hunters, trappers and oilfield workers.
 - Support for prohibition on translocation or rehabilitation of wildlife from the enzootic region

Conclusions

- Frequency of indeterminant and false positive results in bats when using the aspiration technique decreases the cost/benefit of preserving skull for archival purposes
 - RT-PCR likely a better test when an intact brain suitable for DFG is not available from a bat
- Indeterminant result rates were high for bears

Acknowledgments

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