CANADIAN WILDLIFE HEALTH COOPERATIVE

CREATING A WORLD
THAT IS SAFE AND SUSTAINABLE
FOR WILDLIFE AND SOCIETY

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Using Laboratory Techniques to Answer Wildlife Disease Questions

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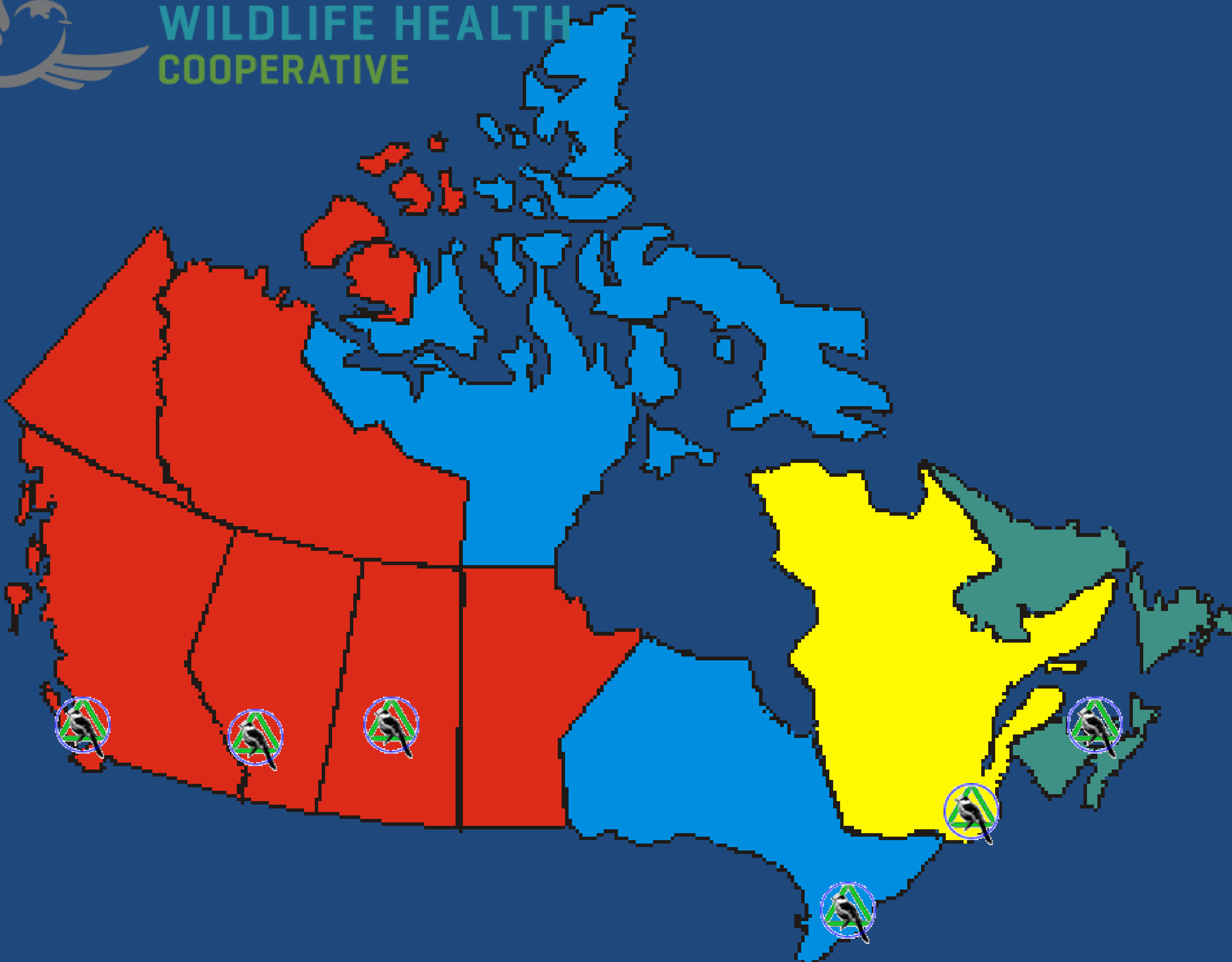


Wildlife Disease Surveillance and Laboratory Testing

- The challenges of wildlife disease diagnosis and surveillance
- The importance of laboratory testing
- The need for exploratory testing and the development of new tests for new pathogens
- The critical role played by more specific tests in targeted or enhanced surveillance for specific diseases

Acknowledgements

- Bacteriology: Marie Archambault, Durda Slavic, Peggy-Jo Osmond
- Virology: Davor Ojkic, Susy Carman
- Toxicology: Brent Hoff, Nick Schrier, Wilson Rumbeiha (Michigan State)
- Molecular Biology: Hugh Cai
- Immunohistochemistry: Josepha Delay, Susan Lapos

The Process of Surveillance

- Observation/Detection
- Reporting to veterinarian/laboratory
- Collection and submission of samples
- Testing at the laboratory
- Diagnosis
- Reporting of results
- Archiving results/database

Components of Domestic Animal Surveillance – Disease Detection and Reporting

- Production unit (farm/barn/pasture)
- Farmer/farm worker
- Veterinarian
- Slaughterhouse

Characteristics of Wildlife Disease Surveillance

- Dispersed population with few boundaries
- Population numbers are unknown
- Lack of trained observers
- Little/no veterinary involvement
- No one is responsible for detection or collection/submission of samples

Challenges of Wildlife Disease Diagnosis and Surveillance

- Detecting disease events
- Obtaining samples
- Getting samples to the laboratory
- Examination and testing of decomposed and degraded specimens
- Development of new tests for emerging diseases

Wildlife Disease Surveillance

- … is like a dog walking on its hind legs. It is not done well. What is remarkable is that it is done at all.

- Samuel Johnson

Wildlife Disease Questions
What is it?

- Simplest question
- Usually a grossly observable abnormality
- The question most often asked by hunters

Wildlife Disease Questions What Killed These Animals?

- May be an individual animal or a larger scale die-off
- Often an unexpected or unusual mortality
- May be caused by a new or emerging disease

Wildlife Diseases New to Ontario in the past 30 years

- Raccoon rabies
- White Nose Syndrome of Bats
- West Nile Virus
- Newcastle Disease Virus
- Type E Botulism
- Mycoplasma gallisepticum
- Chytrid Fungus
- Snake Fungal Disease

Wildlife Diseases Not Yet Found in Ontario

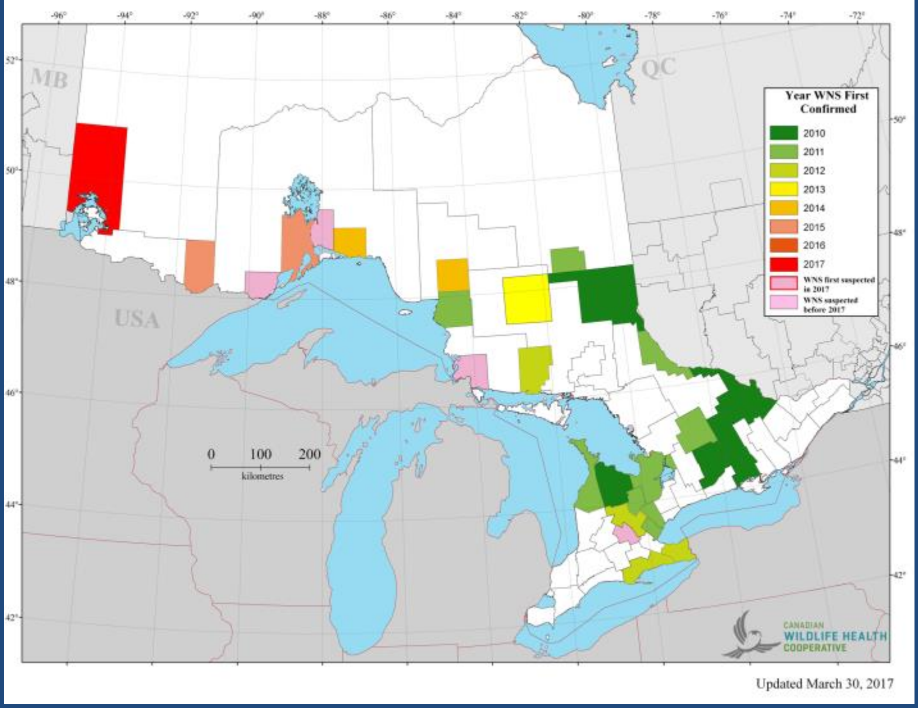
- Chronic Wasting Disease
- Hemorrhagic Disease of White-tailed Deer
- Tuberculosis in White-tailed Deer
- Highly Pathogenic Avian Influenza Virus
- Lymphoproliferative Disease Virus of Wild Turkeys
- Chytrid fungus in salamanders

Answering Open-Ended Questions Requires Exploratory Testing

- These sorts of wildlife disease questions require exploratory testing, which might include:
- Pathology – gross and microscopic
- Microbiology – bacterial and fungal culture
- Virology – virus isolation
- Parasitology – fecal flotation
- Toxicology – GC/MS screen
- Electron Microscopy

Wildlife Disease Questions
Descriptive Epidemiology

- Once a disease is known, new questions arise
- How common is this disease?
- Is the geographic distribution or prevalence changing?
- This requires a different type of laboratory test – e.g. PCR



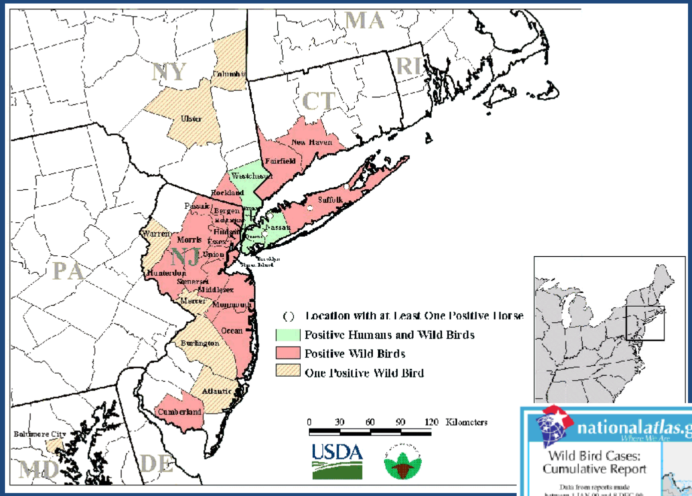
Specific Laboratory Testing and Enhanced Surveillance

- Sensitive test methods, such as PCR, that work even on degraded samples, are ideal for enhanced surveillance for specific pathogens
- When trying to describe the occurrence of a specific disease, extra effort can be made to obtain samples and test them for the presence or absence of that agent
- E.g. current rabies surveillance, using dRIT

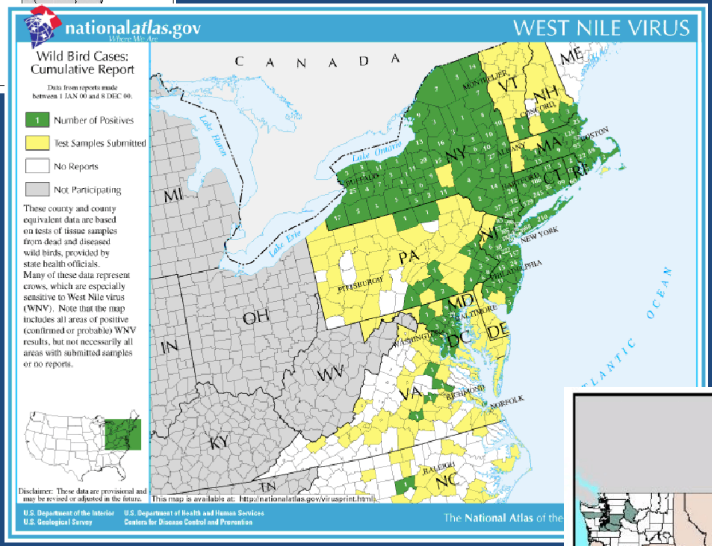
West Nile Virus
A Success Story (sort of)

- WNV was first detected in wild crows in NYC in autumn 1999, co-incident with cases of encephalitis in people
- Disease spread rapidly in North American mosquito and bird populations

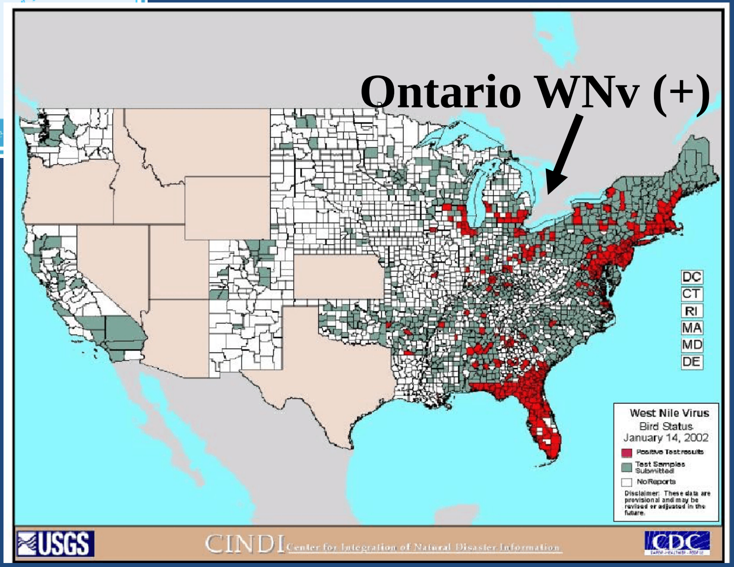
WNV in dead birds, USA 1999-2001



1999

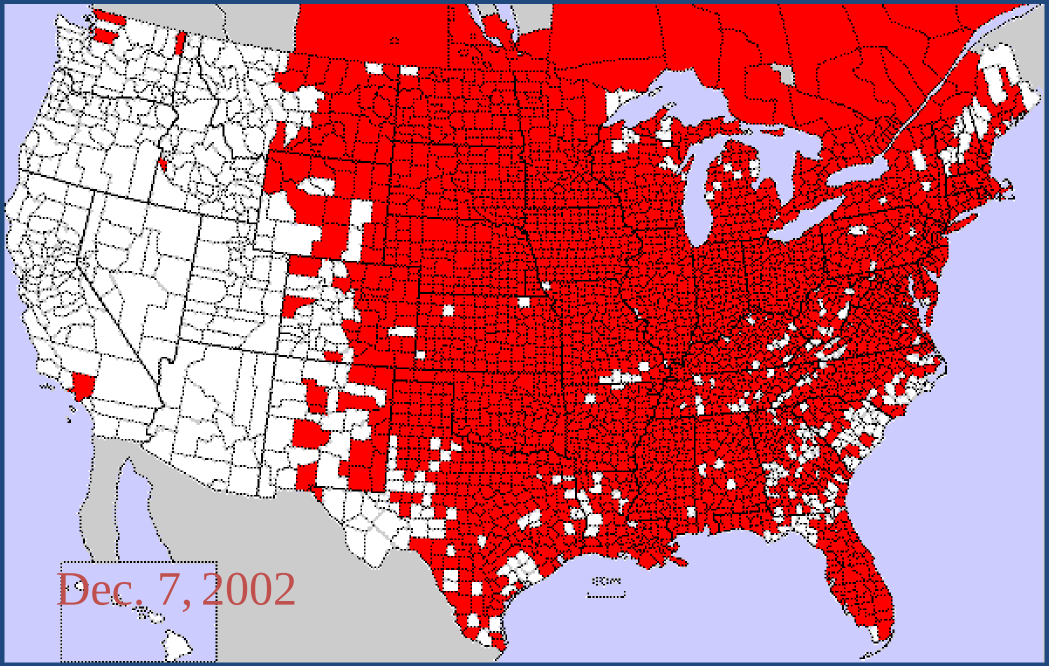


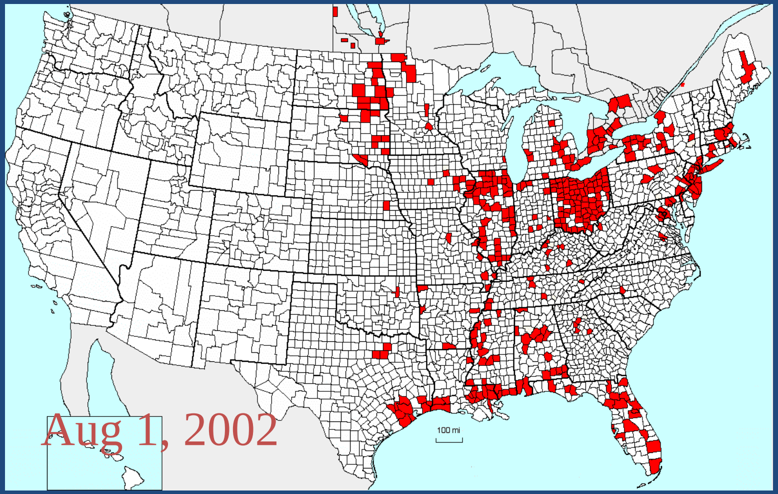
2000

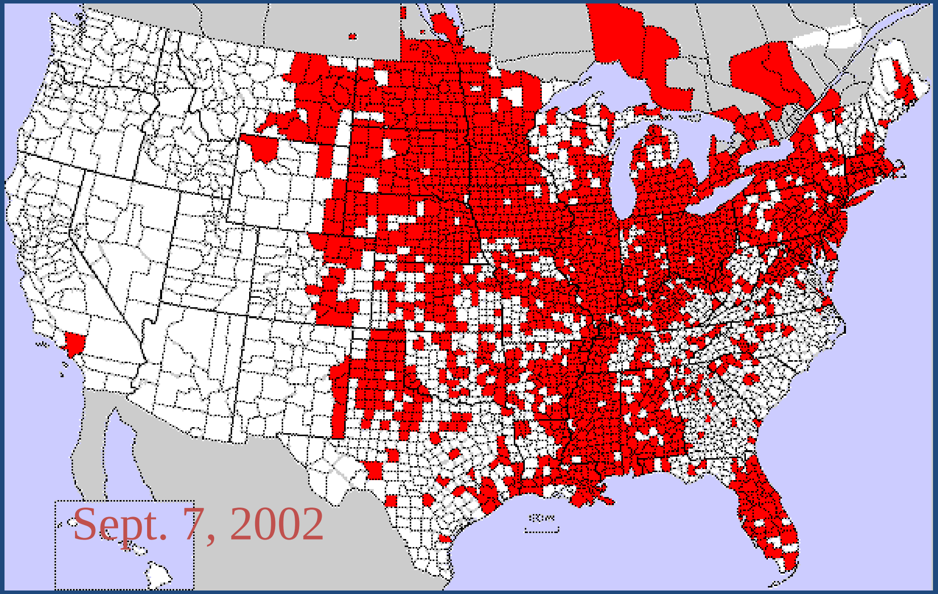


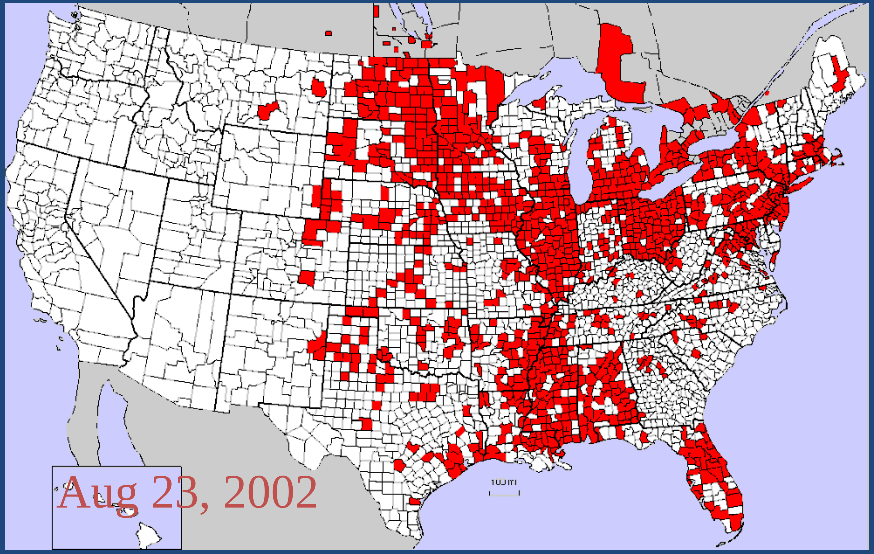
2001

WNV Explosion – 2002









Many humans & horses in eastern NA, including Ontario, horses in Great Plains and Manitoba

Surveillance for WNV in Dead Birds

- Members of the Corvid family (crows, jays, ravens) are uniquely susceptible to WNV
- Testing of dead birds provides an early warning system for the detection of WNV in the environment, earlier than testing of mosquito pools or human cases
- Requires a system of carcass detection, retrieval, submission and testing

CCWHC Dead Bird Processing

- reception of shipping containers with birds
- accession
- triage of unsuitable specimens (wrong species, putrefactive, maggots)

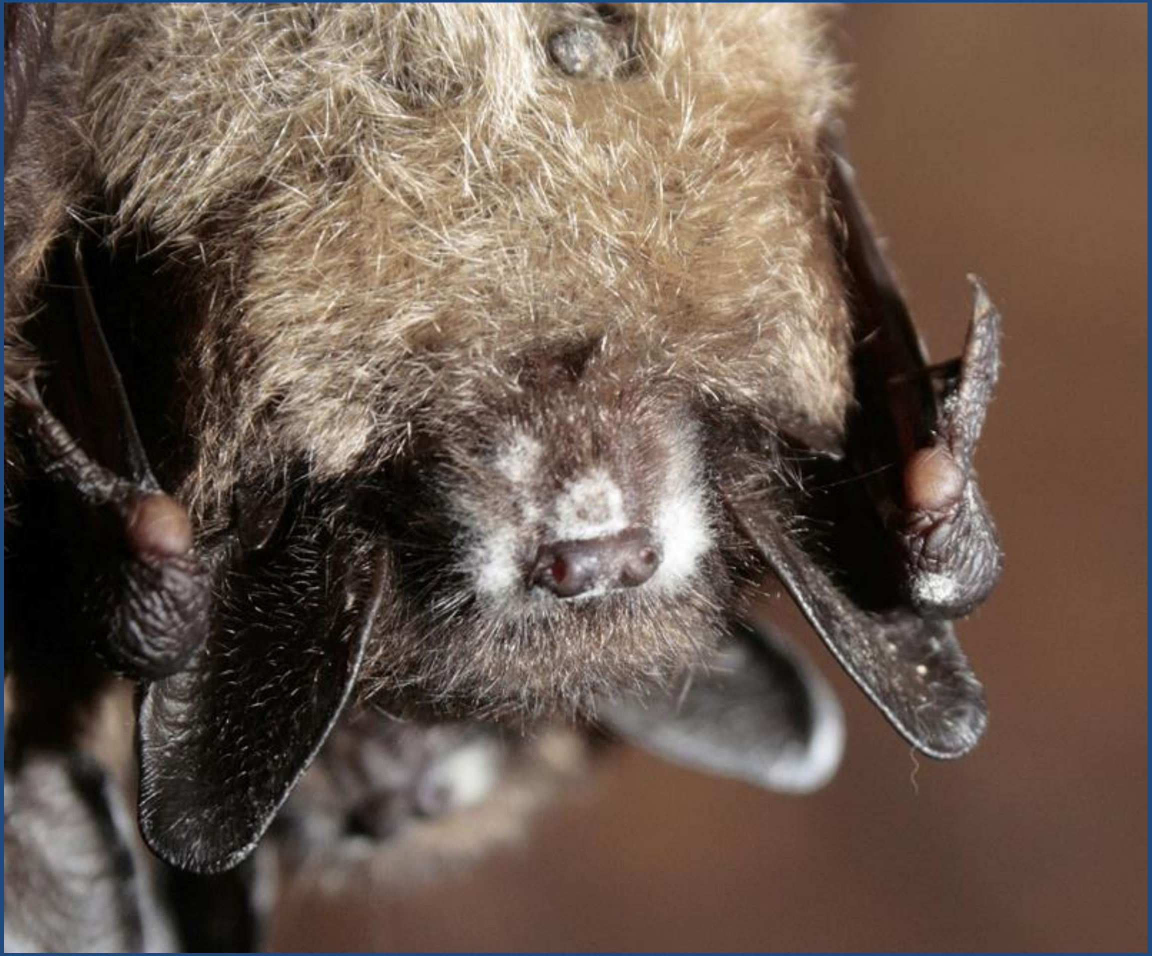
Progression of WNV Test Methods

- In 2000, we relied upon gross and histologic findings. If compatible lesions were found, slides were sent to CFAD lab for IHC
- In 2001-2002, PCR was done in Winnipeg
- From 2003-2005, VecTest (a rapid antigen capture assay) was used; positive test results were confirmed by RT-PCR in Winnipeg
- From 2005, PCR was done at regional centres

Current State of WNV in Canada

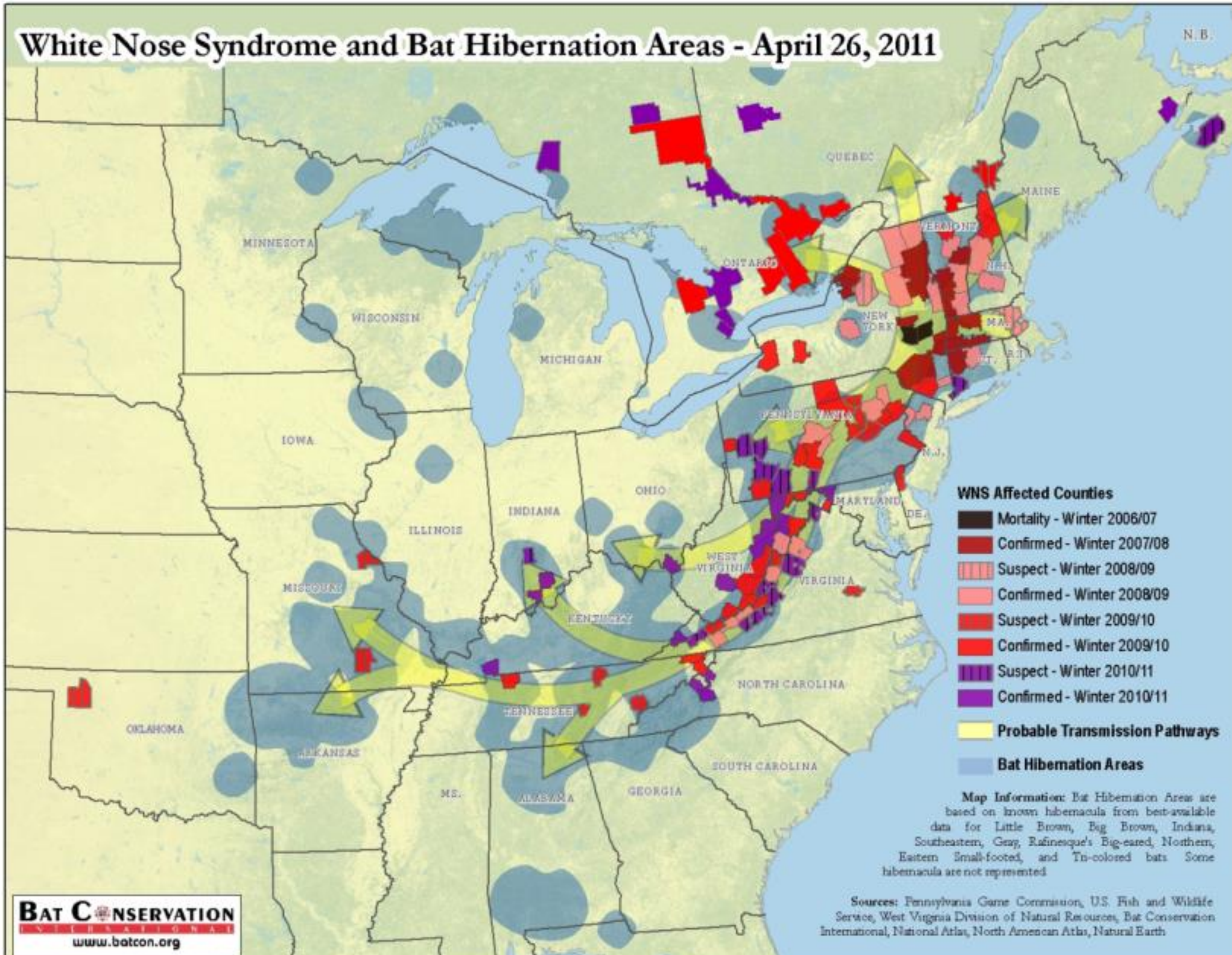
- Routine dead bird surveillance ceased in 2006
- CWHC continues to test dead birds on a smaller scale
- In 2015, 39 of 92 birds tested positive
- Human cases vary widely year to year
- 2007 – 2,215 cases; 2015 – 80 cases
- With climate change, it is expected that the disease will become more widespread

White Nose Syndrome of Hibernating Bats



White Nose Syndrome

- Fungal infection of the epidermis, affecting wings and muzzle – *Pseudogymnoascus destructans*
- Fungus was likely introduced from Europe by a visitor to Howe’s Cavern in NY state
- Disease has caused severe mortality in hibernating bats, leading to 4 species of bats being added to endangered species list

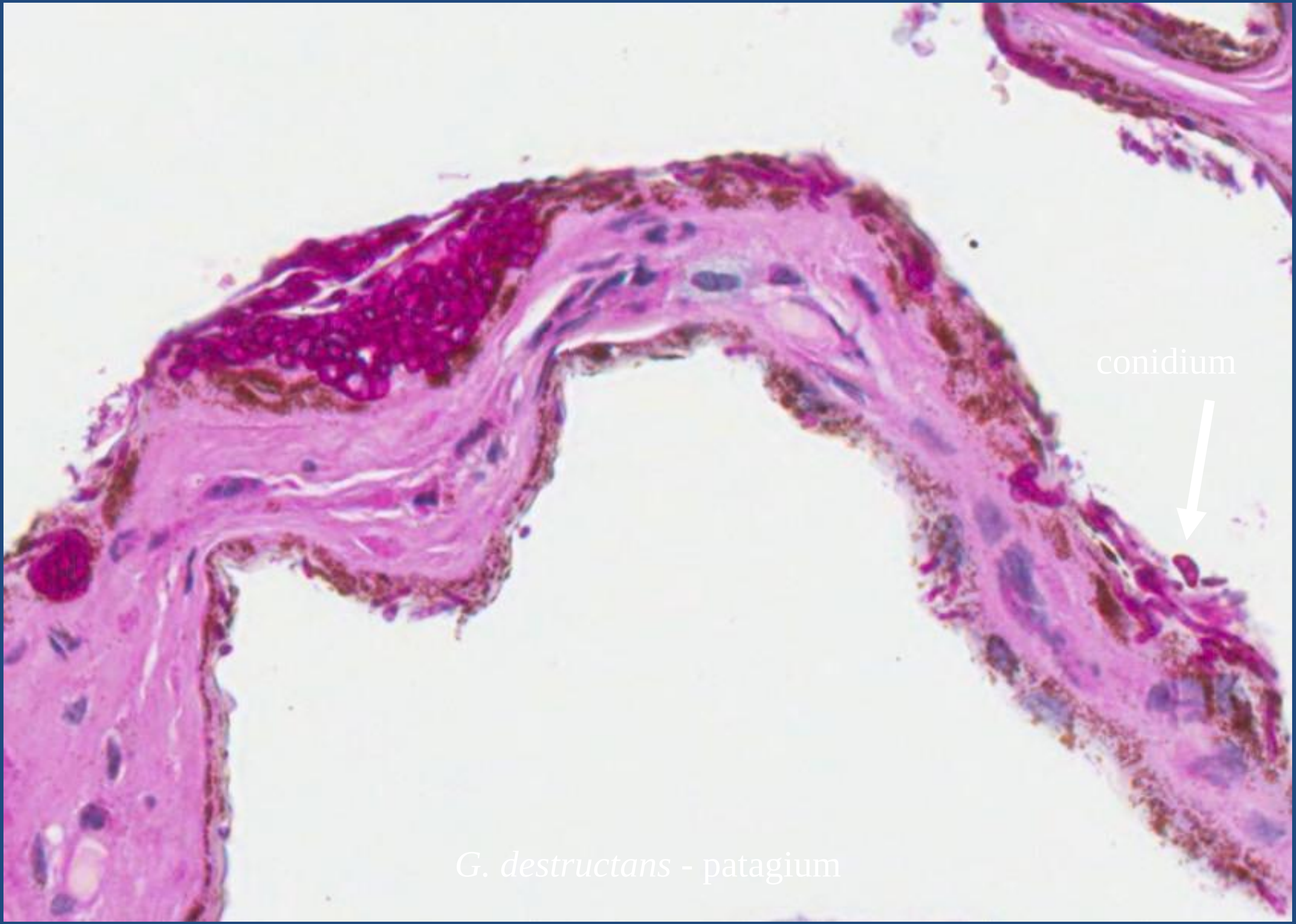


White Nose Syndrome and Bat Hibernation Areas - April 26, 2011
N.B.
QUEBEC
MAINE
VERMONT
N.H.
ONTARIO
NEW YORK
MA.
CT.
R.I.
MINNESOTA
WISCONSIN
MICHIGAN
IOWA
PENNSYLVANIA
N.J.
OHIO
INDIANA
ILLINOIS
MARYLAND
DE.
WEST VIRGINIA
VIRGINIA
MISSOURI
KENTUCKY
NORTH CAROLINA
TENNESSEE
OKLAHOMA
ARKANSAS
SOUTH CAROLINA
MS.
ALABAMA
GEORGIA
WNS Affected Counties
Mortality - Winter 2006/07
Confirmed - Winter 2007/08
Suspect - Winter 2008/09
Confirmed - Winter 2008/09
Suspect - Winter 2009/10
Confirmed - Winter 2009/10
Suspect - Winter 2010/11
Confirmed - Winter 2010/11
Probable Transmission Pathways
Bat Hibernation Areas
Map Information: Bat Hibernation Areas are based on known hibernacula from best-available data for Little Brown, Big Brown, Indiana, Southeastern, Gray, Rafinesque's Big-eared, Northern, Eastern Small-footed, and Tri-colored bats. Some hibernacula are not represented.
Sources: Pennsylvania Game Commission, U.S. Fish and Wildlife Service, West Virginia Division of Natural Resources, Bat Conservation International, National Atlas, North American Atlas, Natural Earth
BAT CONSERVATION INTERNATIONAL
www.batcon.org

Hunt Mine 2010

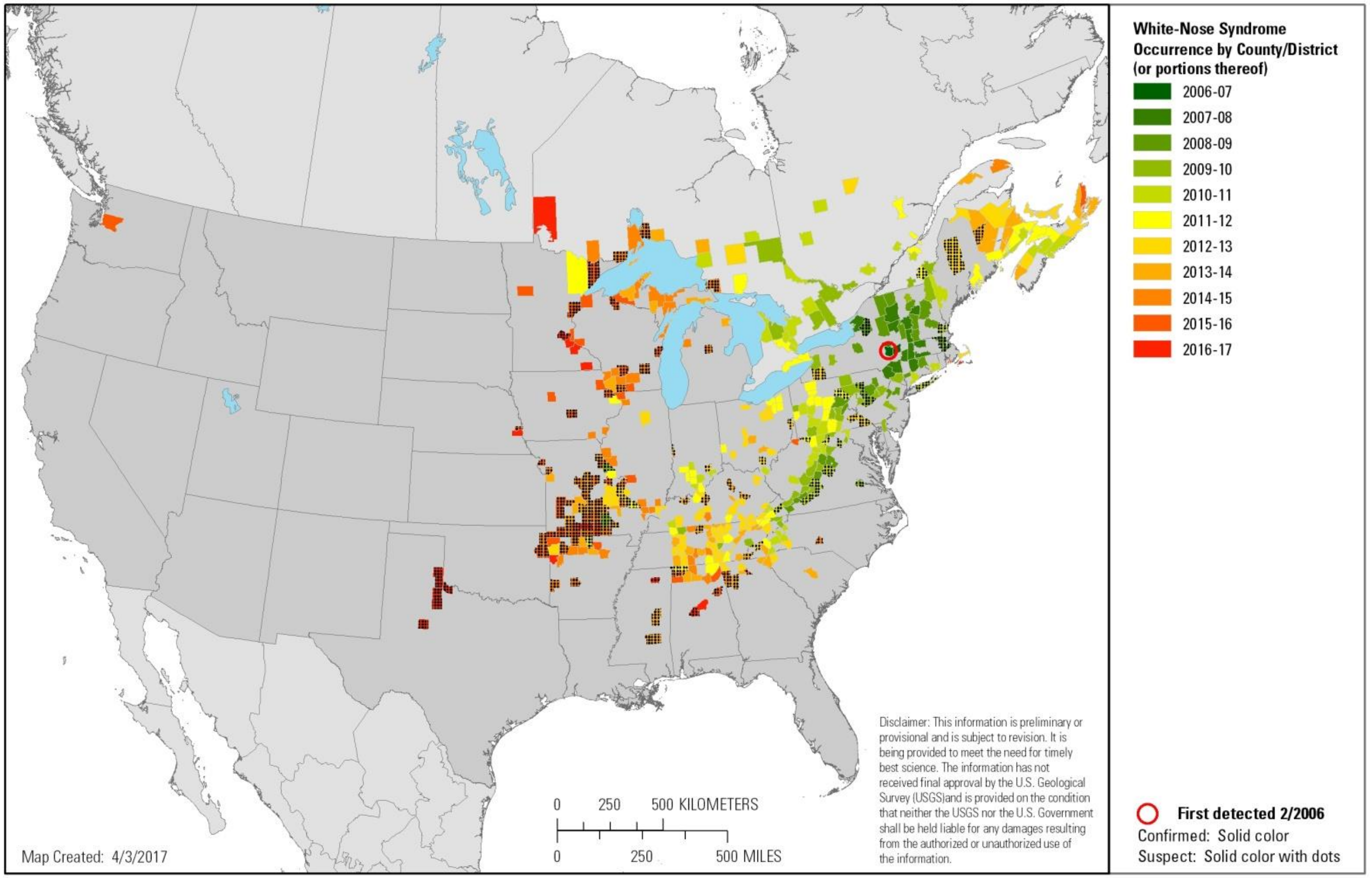
White Nose Syndrome
Progression of Test Methods

- Initially, fungal culture; isolates were sent to NWHC in Madison, Wisconsin for confirmation
- Histological lesions and fungal morphology are distinctive
- Fungal tape lifts were also used
- PCR (usually from tissue swabs) and confirmatory histology are now used



Pink areas in epidermis are *Pseudogymnoasces* hyphae

Current Distribution of White Nose Syndrome



Map depicts the first time WNS is reported suspect or confirmed in a county or district (or portions thereof); each time period in the legend spans a winter bat hibernation period.

Citation: White-nose syndrome occurrence map - by year (2017). Data Last Updated: 4/3/2017. Available at: https://www.whitenosesyndrome.org/resources/map.

Current State of WNS Surveillance

- Restrictions have been placed on entry into hibernacula, particular mines, for reasons of both health and safety and for the protection of the bats
- Bat population numbers are markedly reduced
- Main emphasis in surveillance is in confirming new locations of the disease
- Priority is recovery of bat populations

Type E Botulism

- Marine-associated botulism serotype
- Human disease usually involves fish or marine mammals prepared without cooking
- Disease in wildlife is largely restricted to lower Great Lakes

History of Type E Botulism

- First appeared on Lake Michigan in 1960's
- First appeared in Canadian waters in Lake Huron in 1998
- Has gradually moved northward to Georgian Bay and eastward to eastern Lake Ontario






Diagnosis of Type E Botulism

- Mouse inoculation test remains the most sensitive method
- Ideal sample is serum from a sick bird
- Often liver/heart blood from a dead bird is used
- Must rule out other potential causes of death

Shortcomings of Current Botulism Test Methods

- Ideal sample is usually not available
- Toxin could develop within a carcass after death
- Test is laborious, expensive and uses live animals, requiring stringent protocols
- Test results are often inconclusive and diagnoses may be reported as Suspect/Probable

Canine Distemper Virus Diagnostic Progress and a Cautionary Tale

- CDV is the most common viral disease of raccoons and skunks in Ontario, with cases also occurring in other species such as fisher, mink and marten
- 25 years ago, CDV diagnosis was based primarily upon the identification of consistent microscopic lesions, particularly the presence of inclusion bodies in affected cells

CDV – Diagnostic Progress

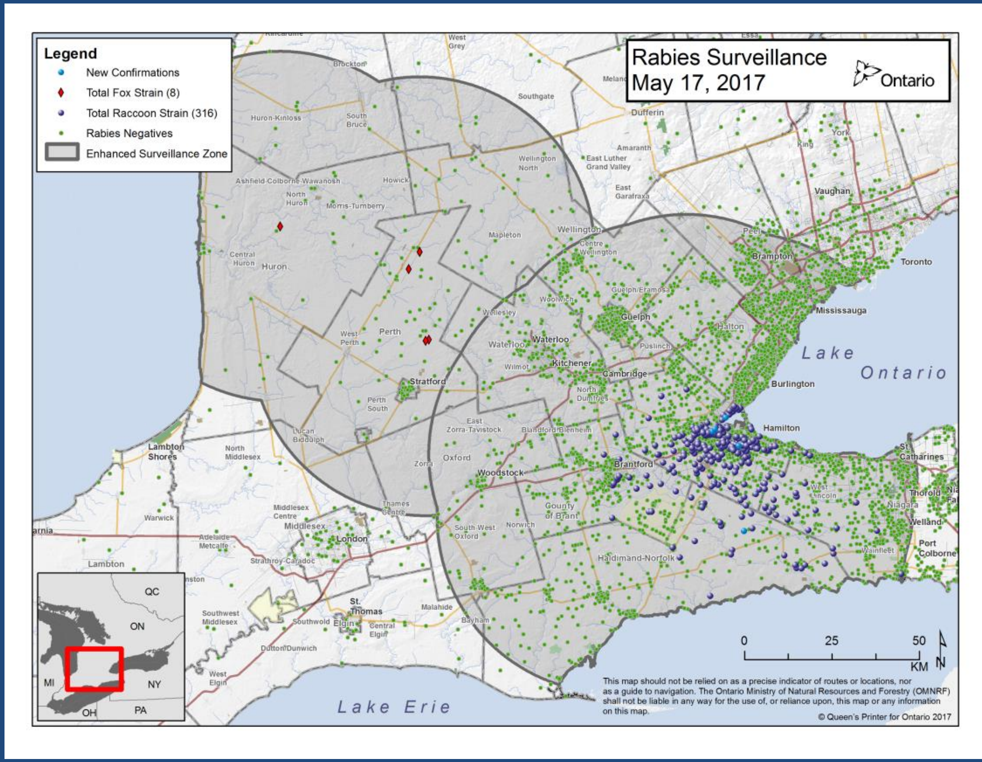
- Immunohistochemistry became available routinely by early 2000's and allowed for confirmation of infection and localization of virus, even in degraded specimens
- PCR was subsequently developed, improving sensitivity even more
- PCR was initially done on lung and mesenteric lymph node

CDV – PCR Diagnosis

- Comparative testing demonstrated that conjunctival swabs were equally sensitive for diagnosis of CDV by PCR
- This would allow for field sampling of affected animals (e.g. by animal control agencies) without the need to submit an entire carcass
- Offered the potential to better map out the distribution of CDV in the province

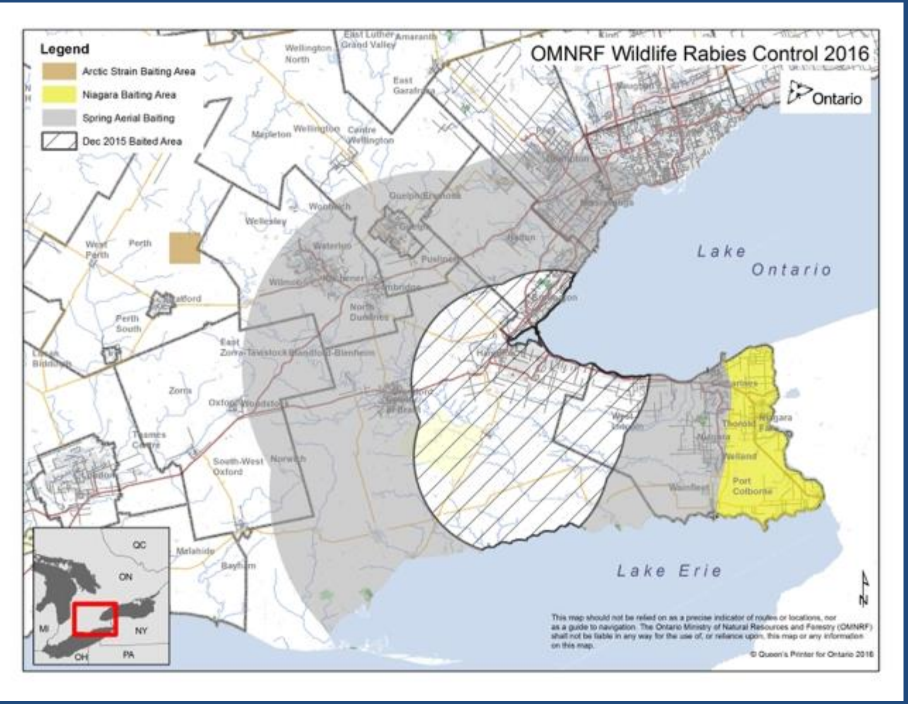
Return of Raccoon Rabies

- In December 2015, raccoon rabies was found in a raccoon from Hamilton, west of the vaccination corridor
- Subsequent intensive surveillance by MNRF revealed that disease had likely been present for some time



Niagara Rabies Vaccination Corridor

- For many years, rabies vector species within this zone had been vaccinated (aerial/hand/TVR)
- The idea was to provide a barrier to the entry of rabies from across the Niagara River



False Confidence in Rabies Vaccination and CDV Diagnosis

- CDV epidemic was ongoing in raccoons in southern Ontario
- In the absence of rabies, it was assumed to be the cause of most cases of neurological disease in raccoons
- It was now possible to diagnosis CDV using PCR on a conjunctival swab with no need to extract and examine the brain

The Risk of Missing Rabies

- Rabies was not missed in any cases submitted to CWHC, but could easily have been
- Subsequent testing of raccoons from within the rabies zone demonstrates that most rabies-negative raccoons are CDV +ve, and co-infection with rabies and CDV is common
- Moral: if you're only looking for one disease, you'll only find one disease

Conclusions

- Laboratory tests such as PCR are effective in testing for specific diseases
- When looking for an unknown, more open-ended tests are needed
- To get the correct answer, you have to ask the right question