

# Sniffing out danger:

Exploring the spillover potential of highly pathogenic avian influenza virus (H5N1) using genomics in BC's wild skunks

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BC Centre for Disease Control  
Provincial Health Services Authority

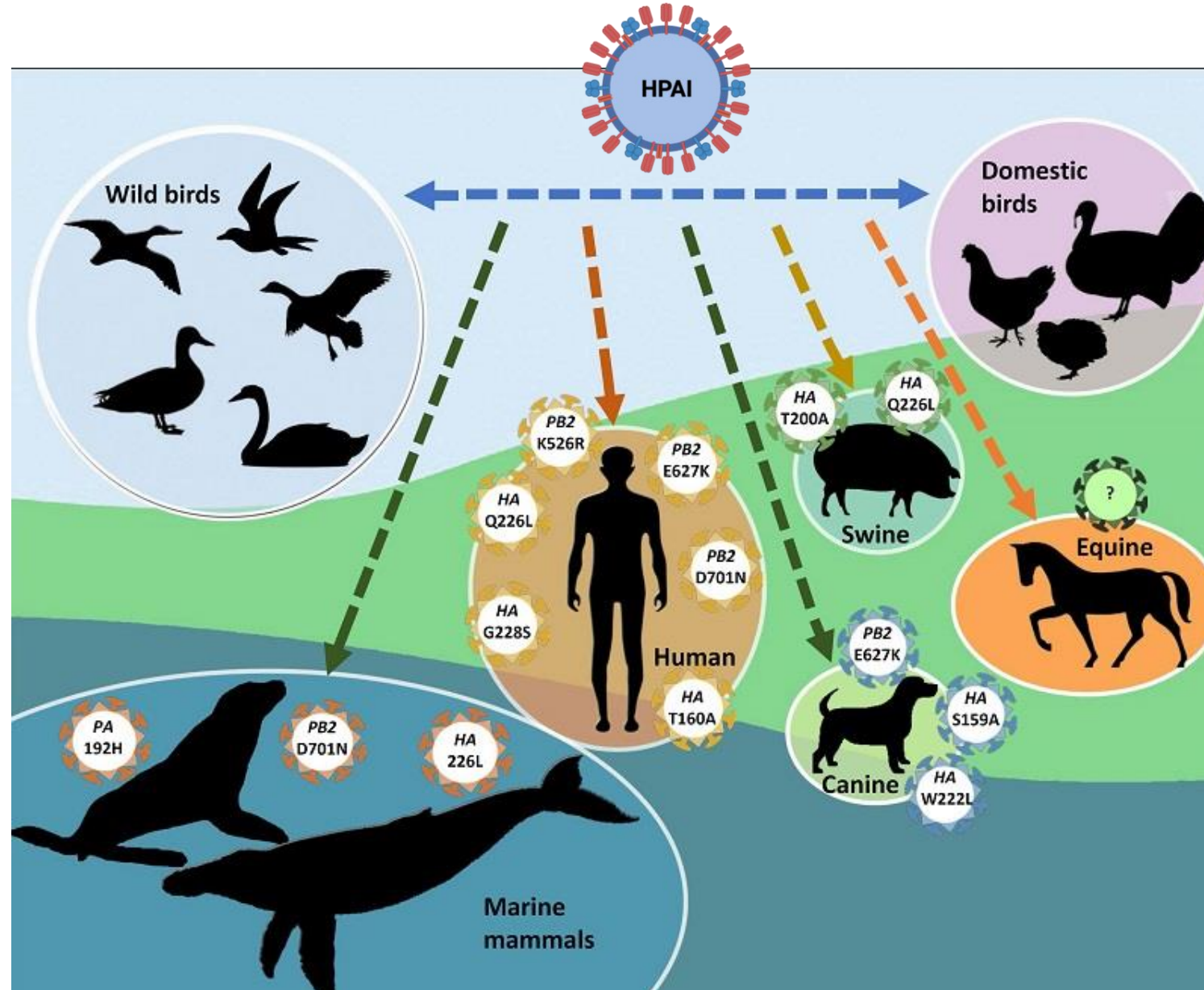


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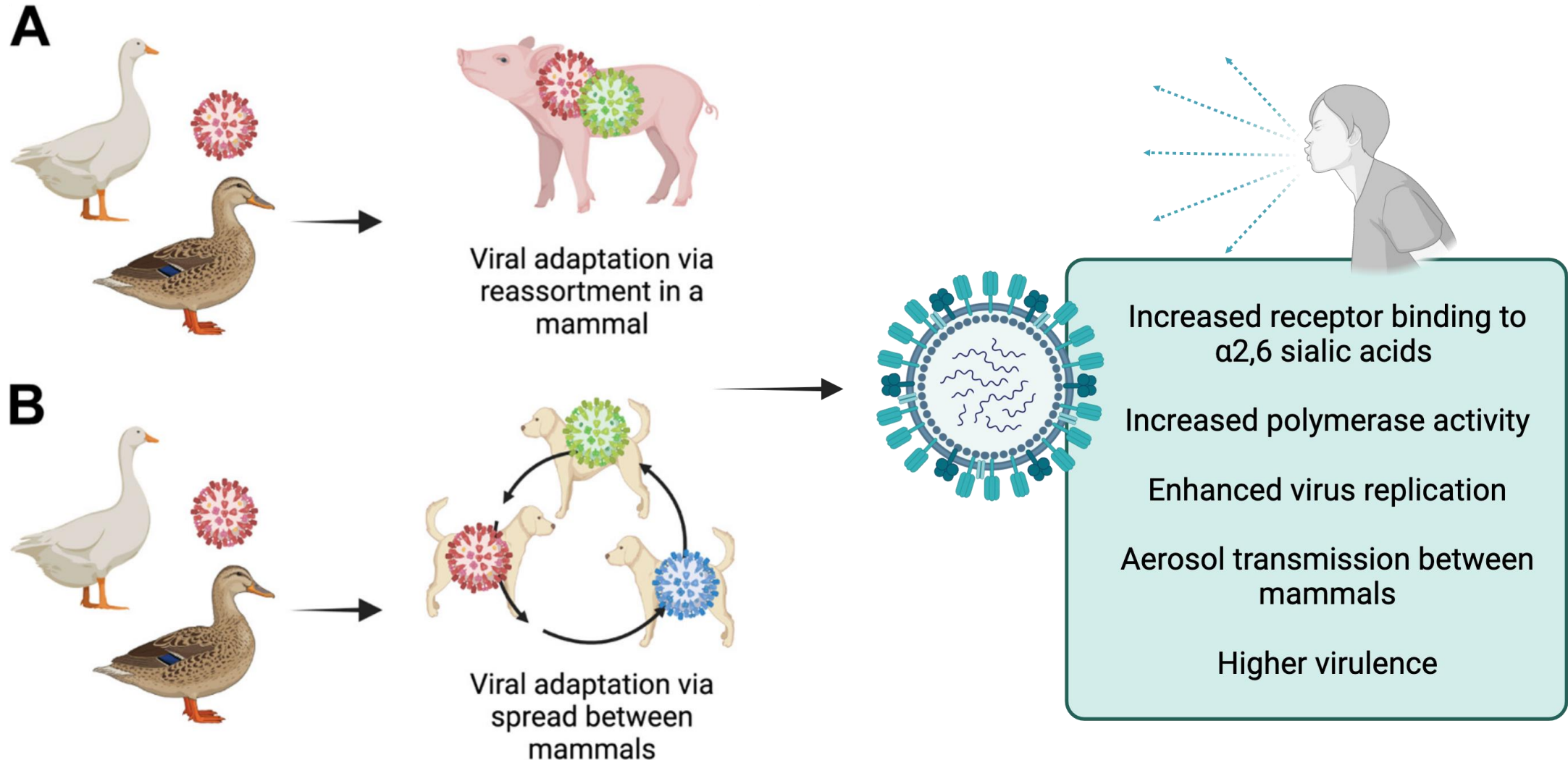
BRITISH  
COLUMBIA

# Highly pathogenic avian influenza spillover into mammals



Lloren, K. et al., *Int. J. Mol. Sci.* **2017**, *18*, 2706.





# Highly pathogenic avian influenza spillover into mammals occurs by **adaptation**



# Numerous isolated spillover events in mammals have been identified globally, increasing concern over H5N1's "pandemic potential"

Rapid communication

## Highly pathogenic avian influenza A(H5N1) virus infection in farmed minks, Spain, October 2022

Montserrat Agüero<sup>1,\*</sup>, Isabella Monne<sup>2\*</sup> , Azucena Sánchez<sup>1</sup>, Bianca Zecchin<sup>2</sup> , Alice Fusaro<sup>2</sup> , María José Ruano<sup>1</sup>, Manuel del Valle Arrojo<sup>3</sup>, Ricardo Fernández-Antonio<sup>4</sup> , Antonio Manuel Souto<sup>5</sup>, Pedro Tordable<sup>5</sup>, Julio Cañas<sup>5</sup>, Francesco Bonfante<sup>2</sup>, Edoardo Giussani<sup>2</sup>, Calogero Terregino<sup>2</sup>, Jesús Javier Orejas<sup>6</sup>

 | Open Peer Review | Virology | Research Article | 23 January 2023

## Highly Pathogenic Avian Influenza H5N1 Virus Infections in Wild Red Foxes (*Vulpes vulpes*) Show Neurotropism and Adaptive Virus Mutations

Authors: Luca Bordes , Sandra Vreman, Rene Heutink, Marit Roose, Sandra Venema, Sylvia B. E. Pritz-Verschuren, Jolianne M. Rijks, José L. Gonzales , Evelien A. Germeraad, Marc Engelsma, Nancy Beerens   | [AUTHORS INFO & AFFILIATIONS](#)


## Peru confirms H5N1 avian flu in marine mammals, part of southward spread

*Lisa Schnirring*, February 7, 2023

Topics: [Avian Influenza \(Bird Flu\)](#)

Research Letter

## Highly Pathogenic Avian Influenza A(H5N1) Virus in a Harbor Porpoise, Sweden

Elina Thorsson, Siamak Zohari, Anna Roos, Fereshteh Banihashem, Caroline Bröjer, and Aleksija Neimanis 

Author affiliations: National Veterinary Institute (SVA), Uppsala, Sweden (E. Thorsson, S. Zohari, F. Banihashem, C. Bröjer, A. Neimanis);

Swedish Museum of Natural History, Stockholm, Sweden (A. Roos)

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DISPATCHES

## Highly Pathogenic Avian Influenza A(H5N1) Virus Outbreak in New England Seals, United States

Wendy Puryear<sup>1</sup>, Kaitlin Sawatzki<sup>1</sup>, Nichola Hill, Alexa Foss, Jonathon J. Stone, Lynda Doughty, Dominique Walk, Katie Gilbert, Maureen Murray, Elena Cox, Priya Patel, Zak Mertz, Stephanie Ellis, Jennifer Taylor, Deborah Fauquier, Ainsley Smith, Robert A. DiGiovanni Jr., Adriana van de Guchte, Ana Silvia Gonzalez-Reiche, Zain Khalil, Harm van Bakel, Mia K. Torchetti, Kristina Lantz, Julianna B. Lenoach, Jonathan Runstadler

> [Infect Genet Evol.](#) 2023 Jul;111:105423. doi: 10.1016/j.meegid.2023.105423. Epub 2023 Mar 6.

## Highly pathogenic avian influenza A (H5N1) virus infections in wild carnivores connected to mass mortalities of pheasants in Finland

Niina Tammiranta<sup>1</sup>, Marja Isomursu<sup>2</sup>, Alice Fusaro<sup>3</sup>, Minna Nylund<sup>2</sup>, Tiina Nokireki<sup>4</sup>, Edoardo Giussani<sup>3</sup>, Bianca Zecchin<sup>3</sup>, Calogero Terregino<sup>3</sup>, Tuija Gadd<sup>4</sup>

Affiliations + expand

PMID: 36889484 DOI: [10.1016/j.meegid.2023.105423](https://doi.org/10.1016/j.meegid.2023.105423)

Free article

Influenza infections

## Characterization of neurotropic HPAI H5N1 viruses with novel genome constellations and mammalian adaptive mutations in free-living mesocarnivores in Canada

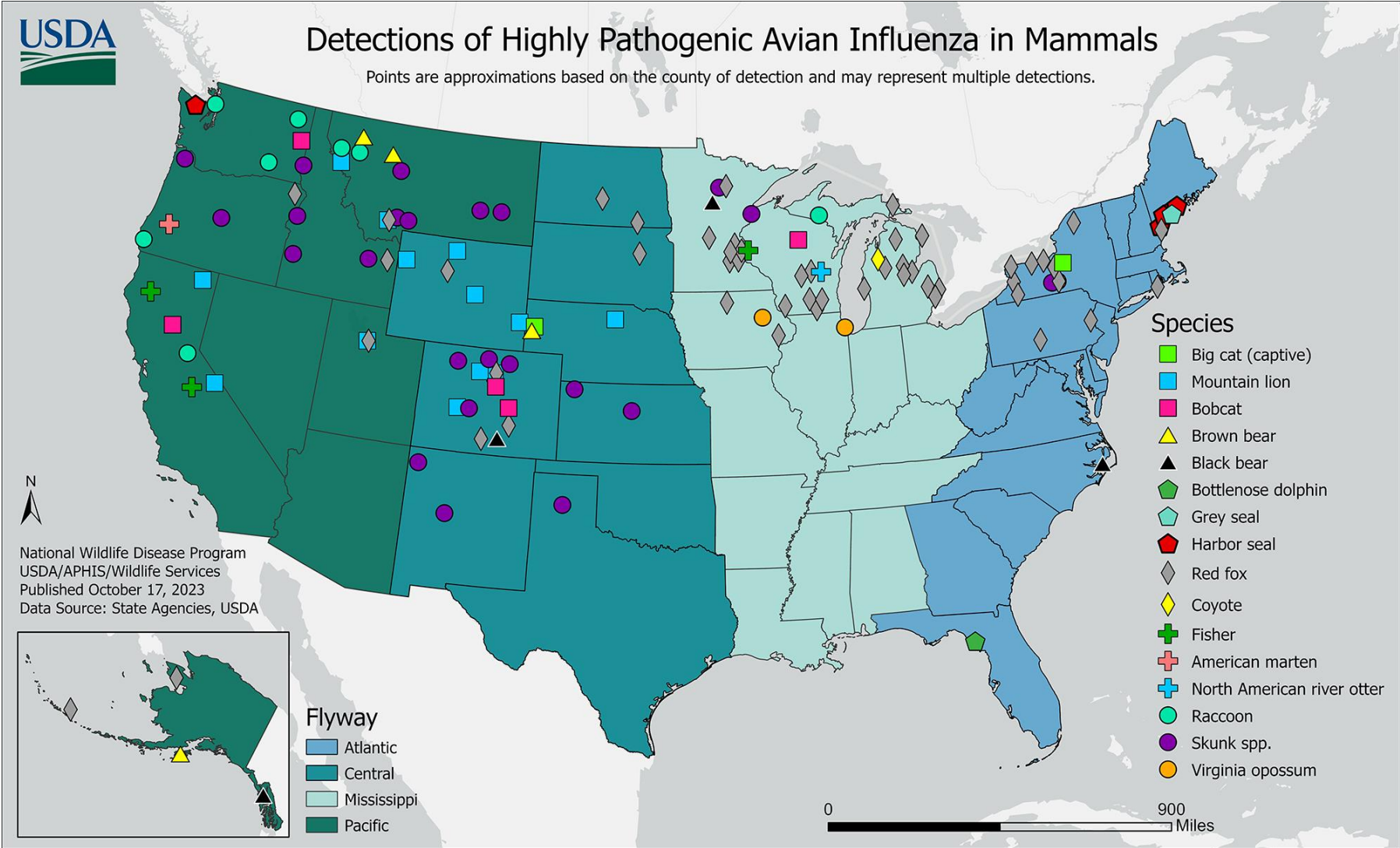
Tamiru N. Alkie, Sherri Cox, Carissa Embury-Hyatt, Brian Stevens, Neil Pople, Margo J. Pybus, ...show all

Article: 2186608 | Received 04 Oct 2022, Accepted 26 Feb 2023, Accepted author version posted online: 07 Mar 2023, Published online: 15 Mar 2023

 Download citation  <https://doi.org/10.1080/22221751.2023.2186608>

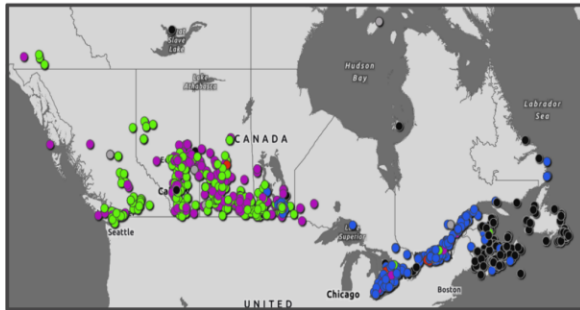
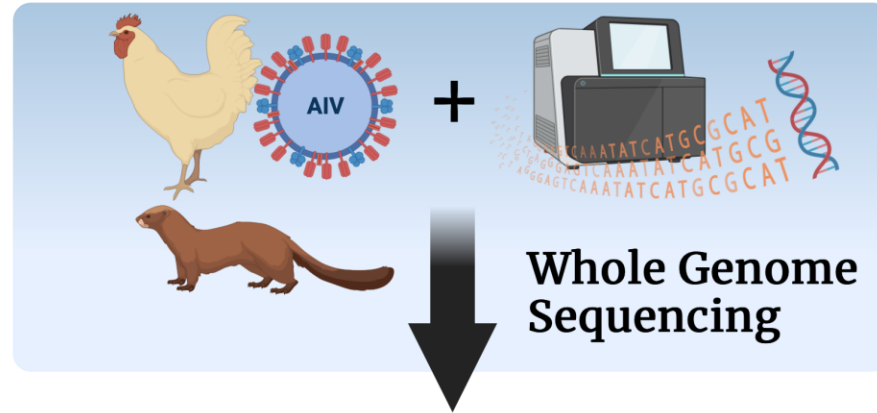
 Check for updates

# USDA: Diverse number of mammal species affected by the HPAI H5N1 (clade 2.3.4.4b) outbreak



How can **genomic** surveillance help us understand the HPAI spillover into mammals?

# Sequencing the genomes of HPAI in mammals and birds provides *molecular evidence* that informs animal and human health risks



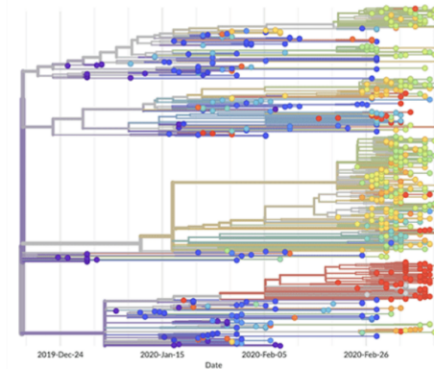
Strains commonly **circulating** in mammals vs. avian species

i.e. How much diversity is out there? Are specific strains correlated with increased mortality?



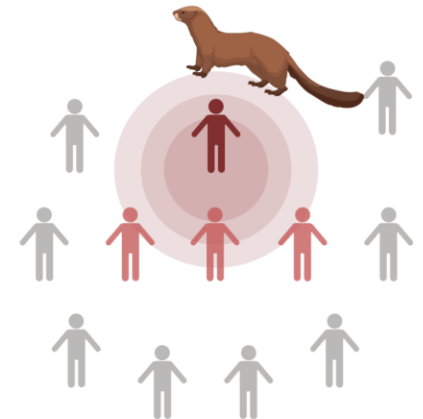
Probable modes of **transmission**

e.g. Transmission between wild birds and mammals, or poultry and mammals



AIV **evolution**

e.g. Are we seeing mutations/reassortments indicative of mammalian adaptation?



**Risk** to human health

e.g. Are we seeing mammal-to-mammal transmission? Has spillover risk increased?

# What about H5N1 in BC mammals?

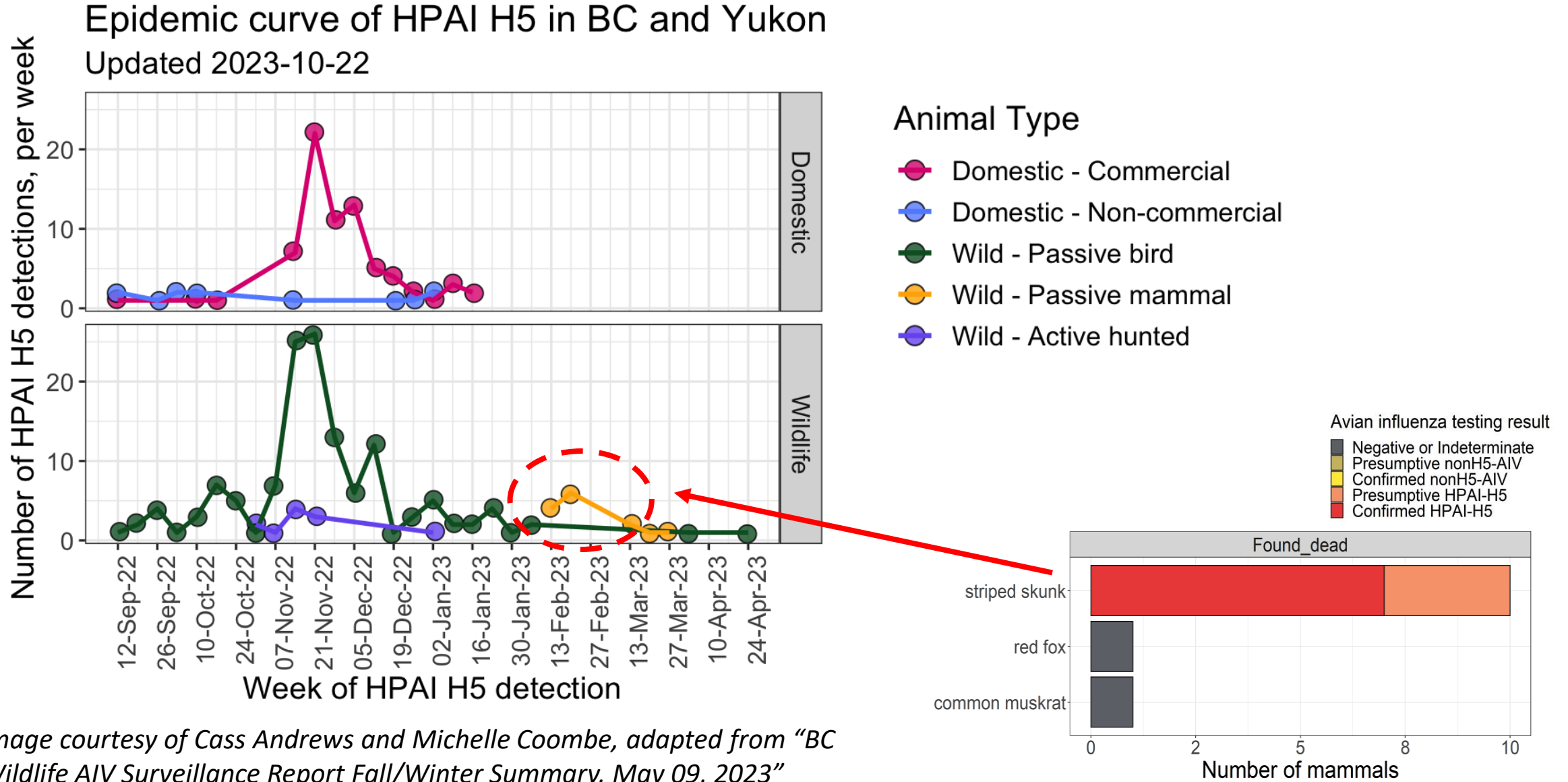


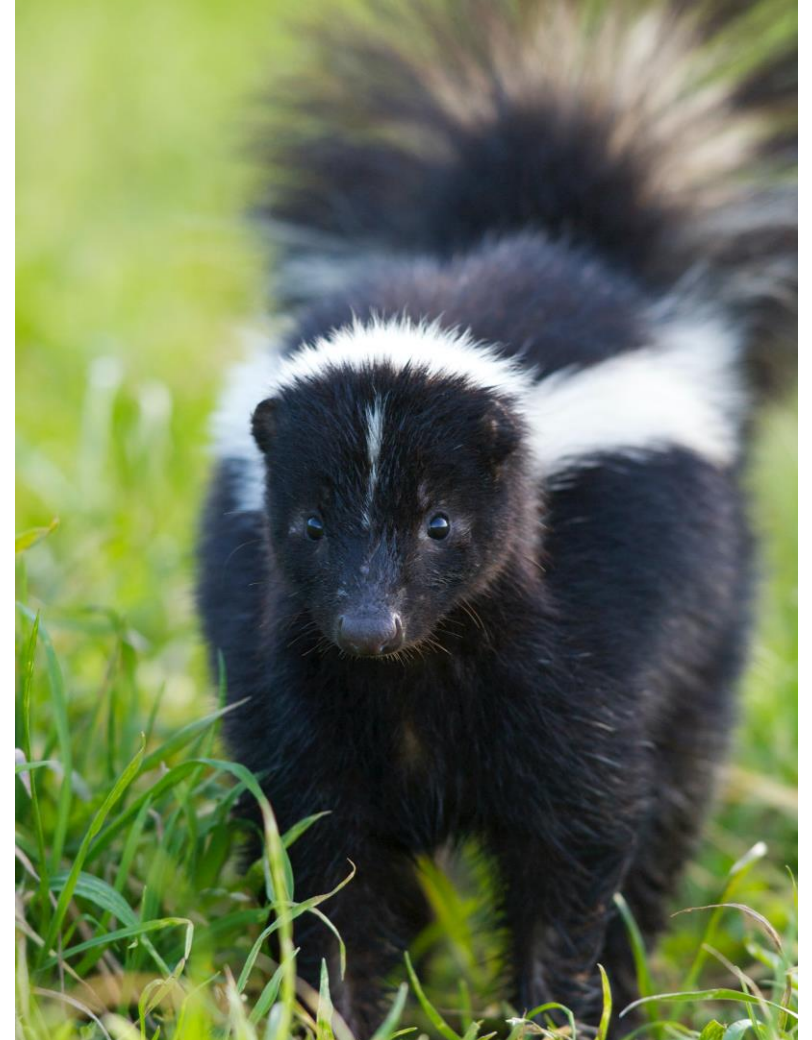
Image courtesy of Cass Andrews and Michelle Coombe, adapted from "BC Wildlife AIV Surveillance Report Fall/Winter Summary, May 09, 2023"



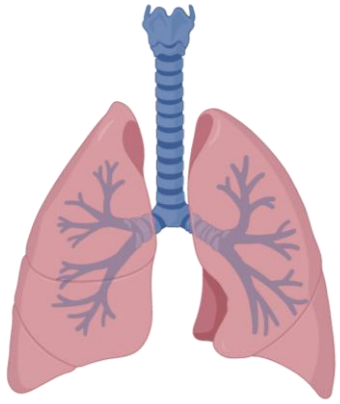
# Between Feb and April 2023, 14 skunks tested positive for H5N1 in BC

- "Wave 1" (April 2022-Sept 2022):
  - Red foxes: 4 positive for H5N1
  - Striped Skunks: 2 positive for H5N1
    - 2 successfully sequenced
- "Wave 2" (Sept 2022-June 2023)
  - Striped skunks: 14 positive for H5N1
    - 12 successfully sequenced
- Post mortem revealed skunks suffered severe pneumonia and meningoencephalitis

*Note:* Specimens from mammals obtained via submissions from wildlife partners (usually Ministry of Forests) from animals that are found sick or dead by the public, or were sent to wildlife rehab

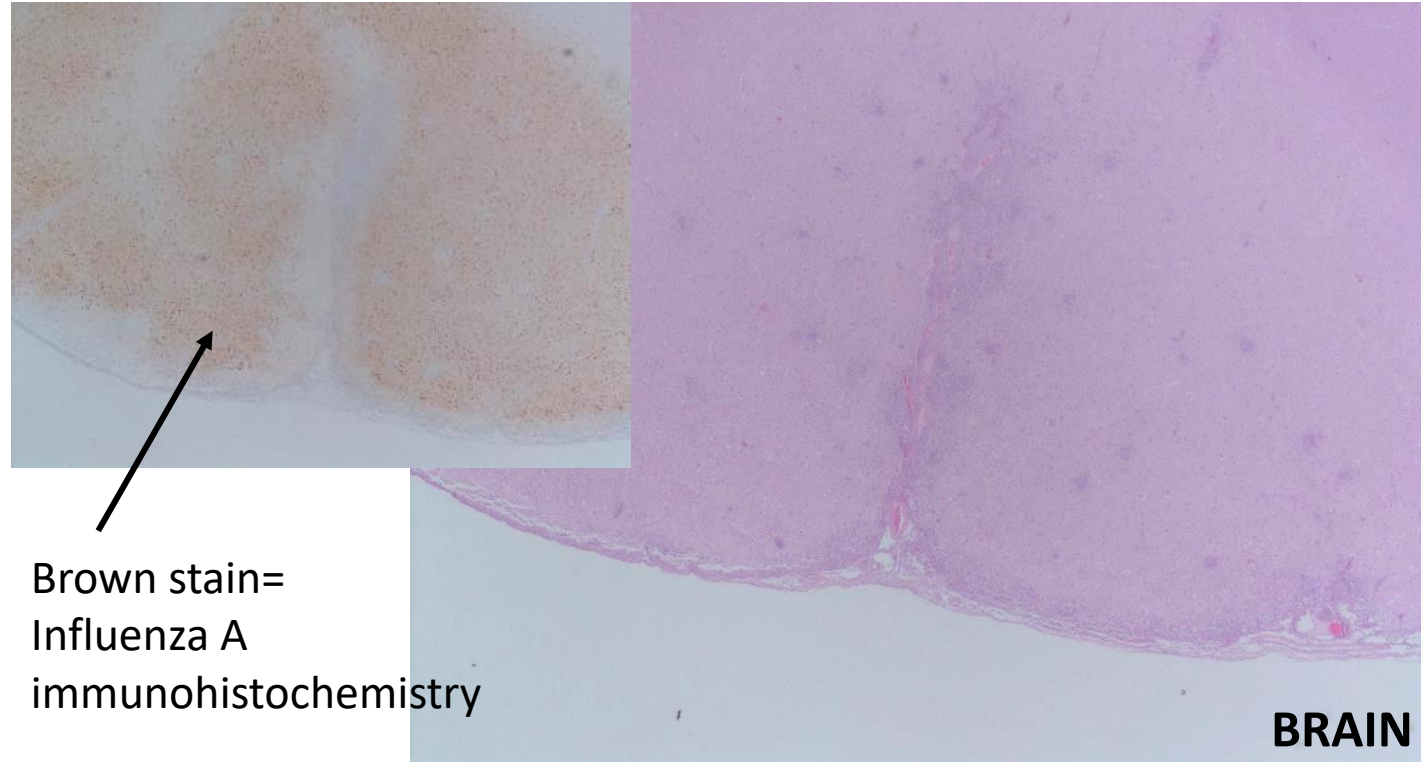
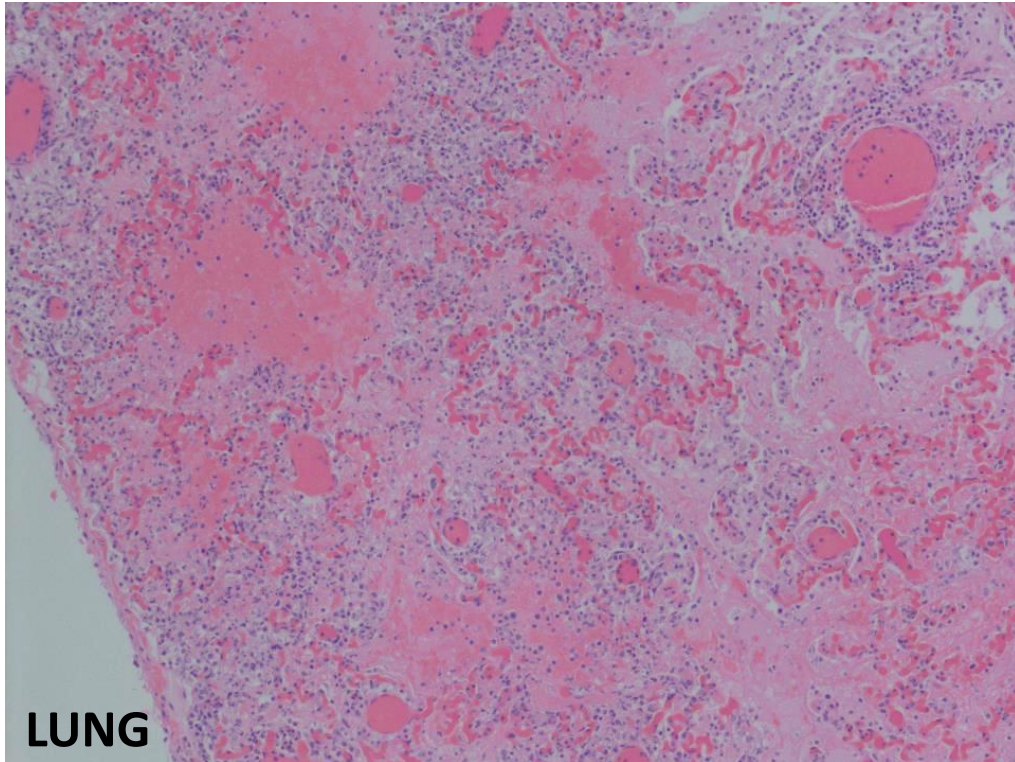


# Striking pathology observed in the infected skunks

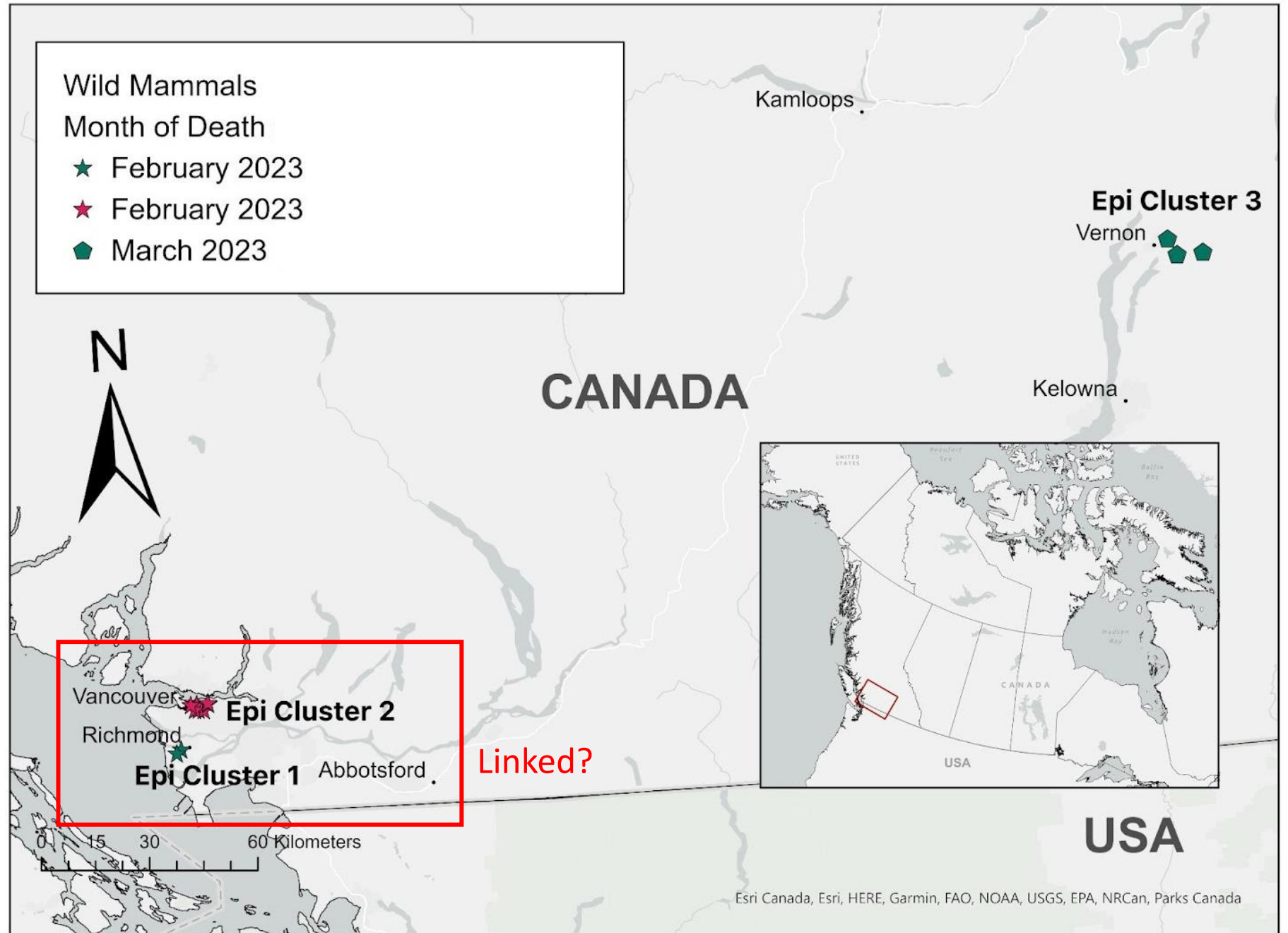


- Multifocal necrotizing interstitial pneumonia
- Severe pathology in the adrenal glands, liver
- Strong Influenza A immunoreactivity in necrotic areas

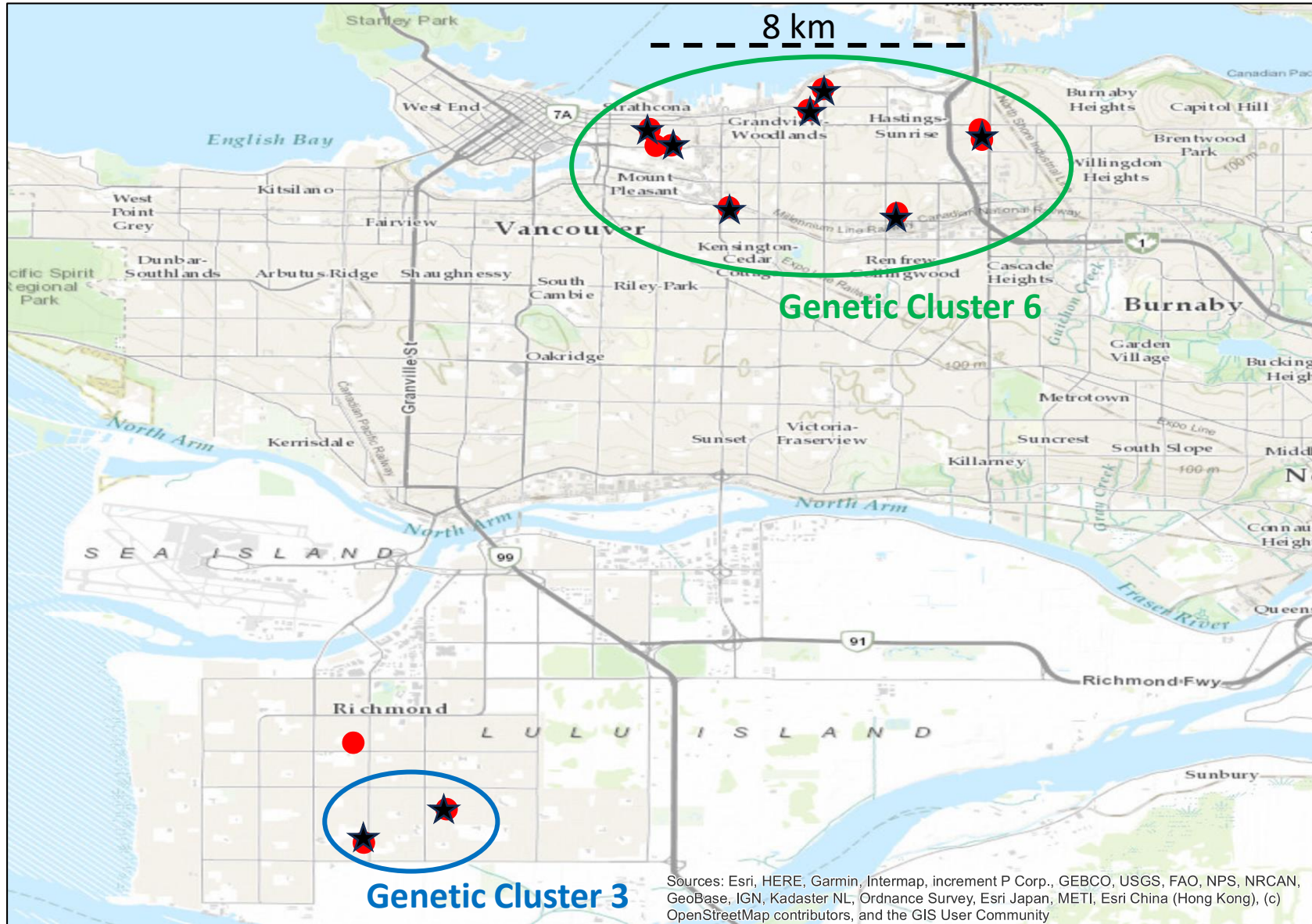
- Extremely severe meningoencephalitis with frequent neuronal necrosis
- High viral load in the brain compared to the lung
- Strong Influenza A immunoreactivity in the neurons of the cerebral cortex



Skunks  
grouped into  
3 distinct  
"epi clusters"



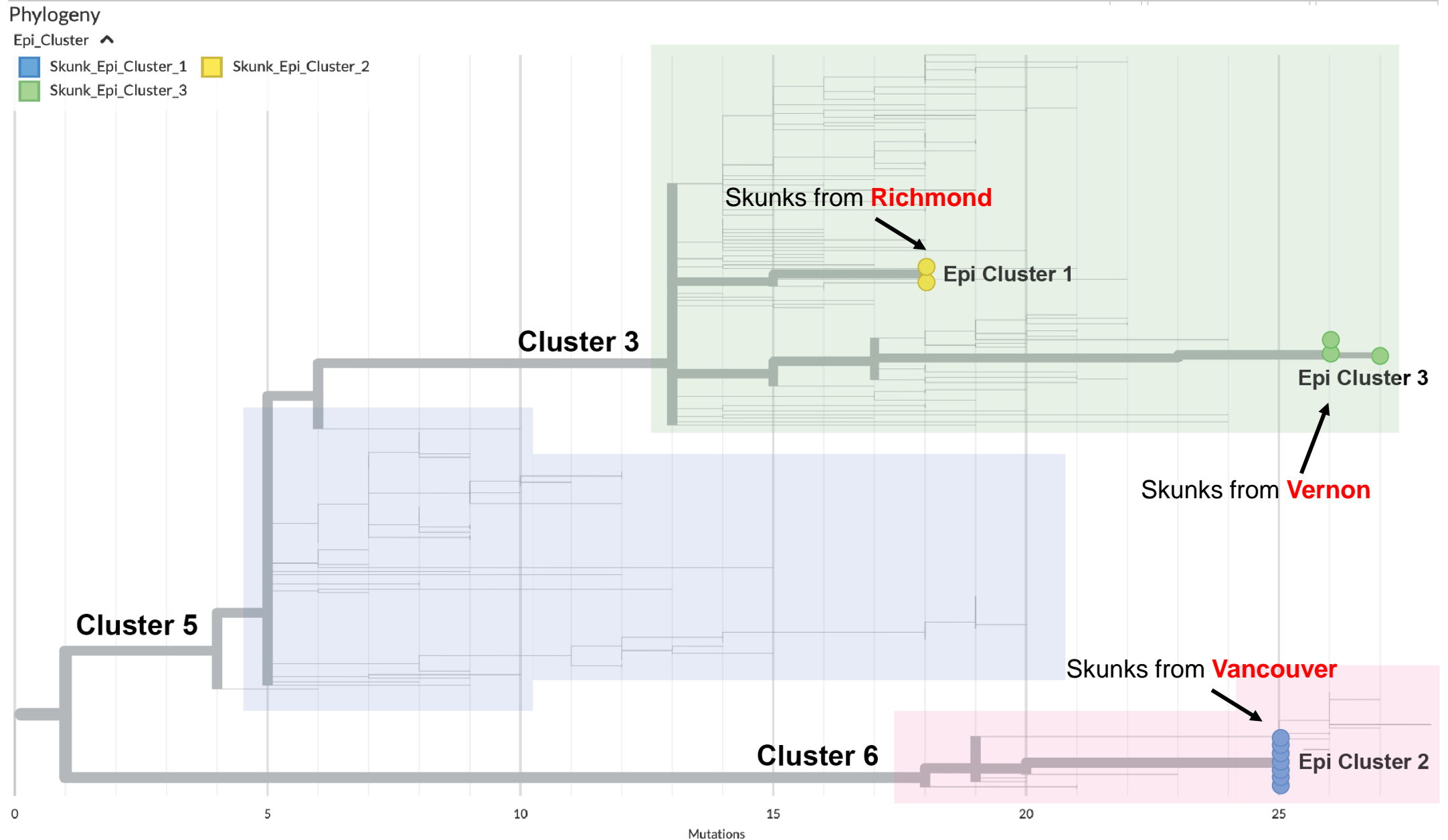
# BC Lower Mainland Skunks: 17 animals found sick or dead Feb '23



- 17 animals retrieved within 10 days
- 10/10 tested positive for HPAI
- 9/10 sequenced successfully
- Vancouver skunks separated by <8km
- Distinct viruses identified in Richmond vs. Vancouver animals

- Animals with GIS coordinates
- ★ Animals with sequence data

# Wild skunk HPAI viruses differ based on *genetics*, geography and time

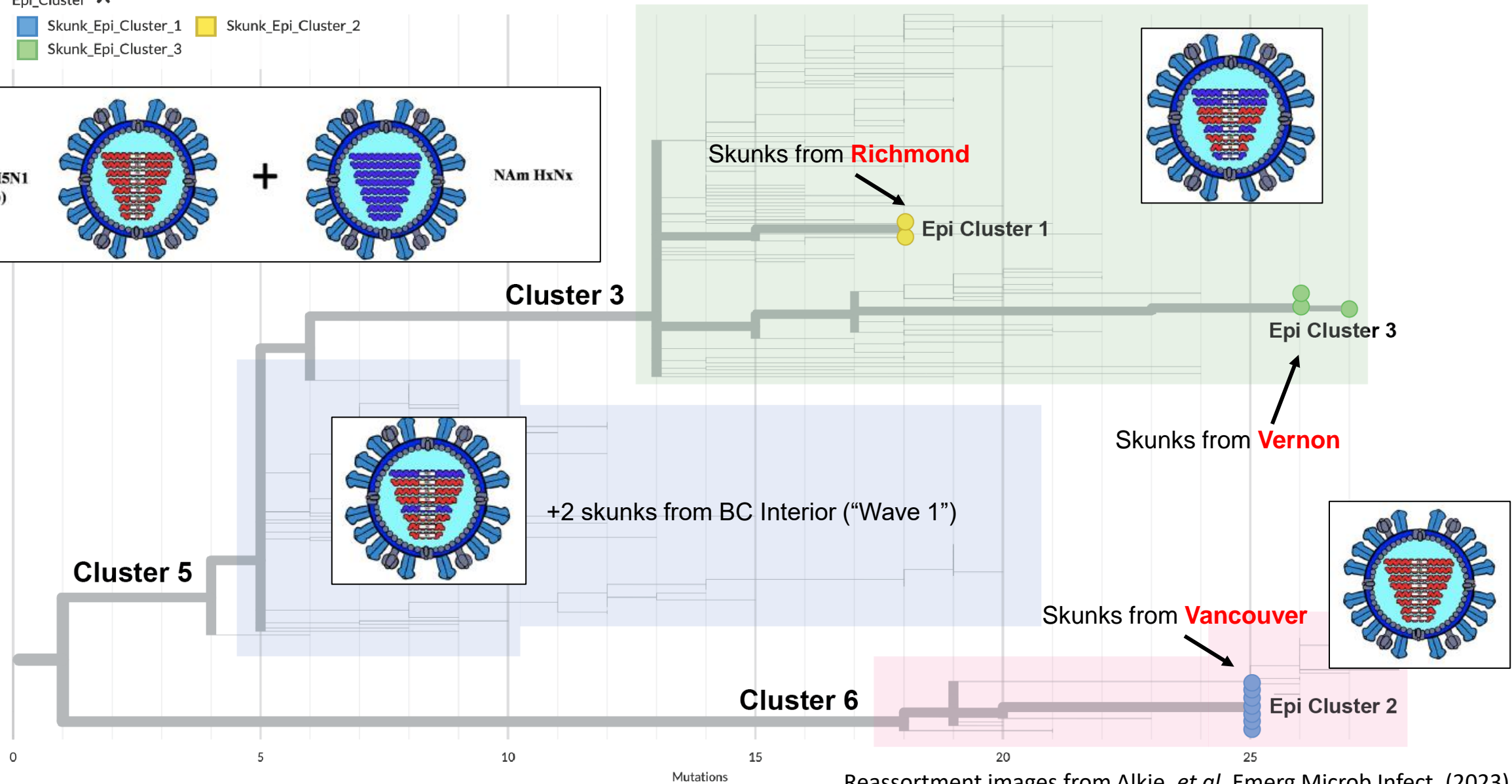
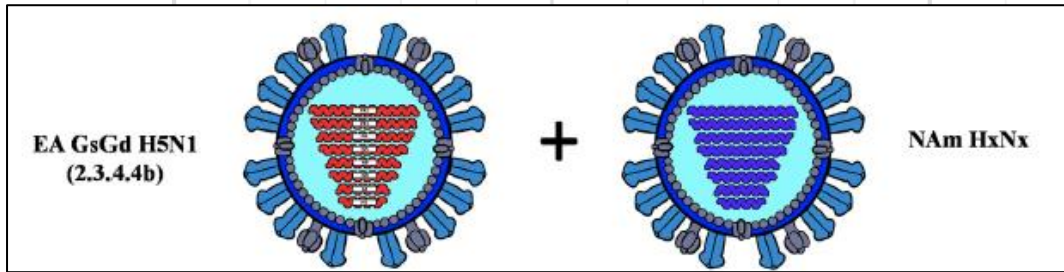


# Wild skunk HPAI viruses represent different genome constellations

Phylogeny

Epi\_Cluster ^

- Skunk\_Epi\_Cluster\_1
- Skunk\_Epi\_Cluster\_2
- Skunk\_Epi\_Cluster\_3



# Monitoring H5N1 sequences for mammalian adaptations

- The majority of key mutations are found in both BC avian and skunk samples, or neither
- There are a few differences that should be monitored:
  - PB2- D701N
  - PB2- E627K
  - HA- T188I (new avian cluster)
  - HA- V210A
  - NP- N319K
- There could also be novel mutations not yet defined
- Monitor mammal clusters, look for evidence of onward transmission

**Table 1.** Frequency of mammalian adaptive mutations in HPAI H5N1 sequences from wild/domestic avian species and skunks during the outbreak in B.C./Yukon (adapted from the HPAI H5N1 Risk Assessment, May 2022, by Skowronski, D *et al.*)

Gene <sup>^</sup>	Mutation (H5 Numbering)	Presence in B.C./Yukon avian sequences* (N≤374, between 2022-2023)	Presence in B.C. skunks* (N≤12, 2023 <sup>f</sup> )
PB2	T271A	0%	0%
	K526R	0%	0%
	<b>E627K/E627A</b>	<b>0%</b>	<b>11% / 33%</b>
	<b>D701N</b>	<b>0%</b>	<b>56%</b>
	S714R	0%	0%
PB1	D3V	<b>100%</b>	<b>100%</b>
	N105S	0%	0%
	D622G	<b>100%</b>	<b>100%</b>
PA	S37A	<b>100%</b>	<b>100%</b>
	V63I	0%	0%
	K356R	1.5%	0%
	N383D	<b>99%</b>	<b>100%</b>
	N409S	<b>99%</b>	<b>100%</b>
HA	D94S	<b>100%</b>	<b>100%</b>
	S133A	<b>100%</b>	<b>100%</b>
	S154N	<b>100%</b>	<b>100%</b>
	S155D	<b>100%</b>	<b>100%</b>
	T156A	<b>100%</b>	<b>100%</b>
	N182K	0%	0%
	<b>T188I</b>	<b>4%</b>	<b>0%</b>
	K189N	<b>100%</b>	<b>100%</b>
	<b>V210A</b>	<b>92%</b>	<b>42%</b>
	K218Q	100%	100%
	Q222L	0%	0%
	S223R	100%	100%
	G224S	0%	0%
NP	<b>N319K</b>	<b>6%</b>	<b>58%</b>
	E434K	0%	0%
M	I43M	<b>100%</b>	<b>100%</b>
	T215A	<b>100%</b>	<b>100%</b>
NS	80-84DEL	0%	0%
	D92E	0%	0%
	I106M	<b>100%</b>	<b>100%</b>
	C138F	<b>100%</b>	<b>100%</b>
	N30D	<b>100%</b>	<b>100%</b>

\*bold text highlight mutations that are present in ≥99% of contemporary B.C./Yukon avian and skunk sequences; red font highlight mutation frequencies that differ between avian and skunk sequences. Number of sequences analyzed per segment differs due to differences in segment coverage.  
<sup>^</sup>Mutations in neuraminidase were not included.

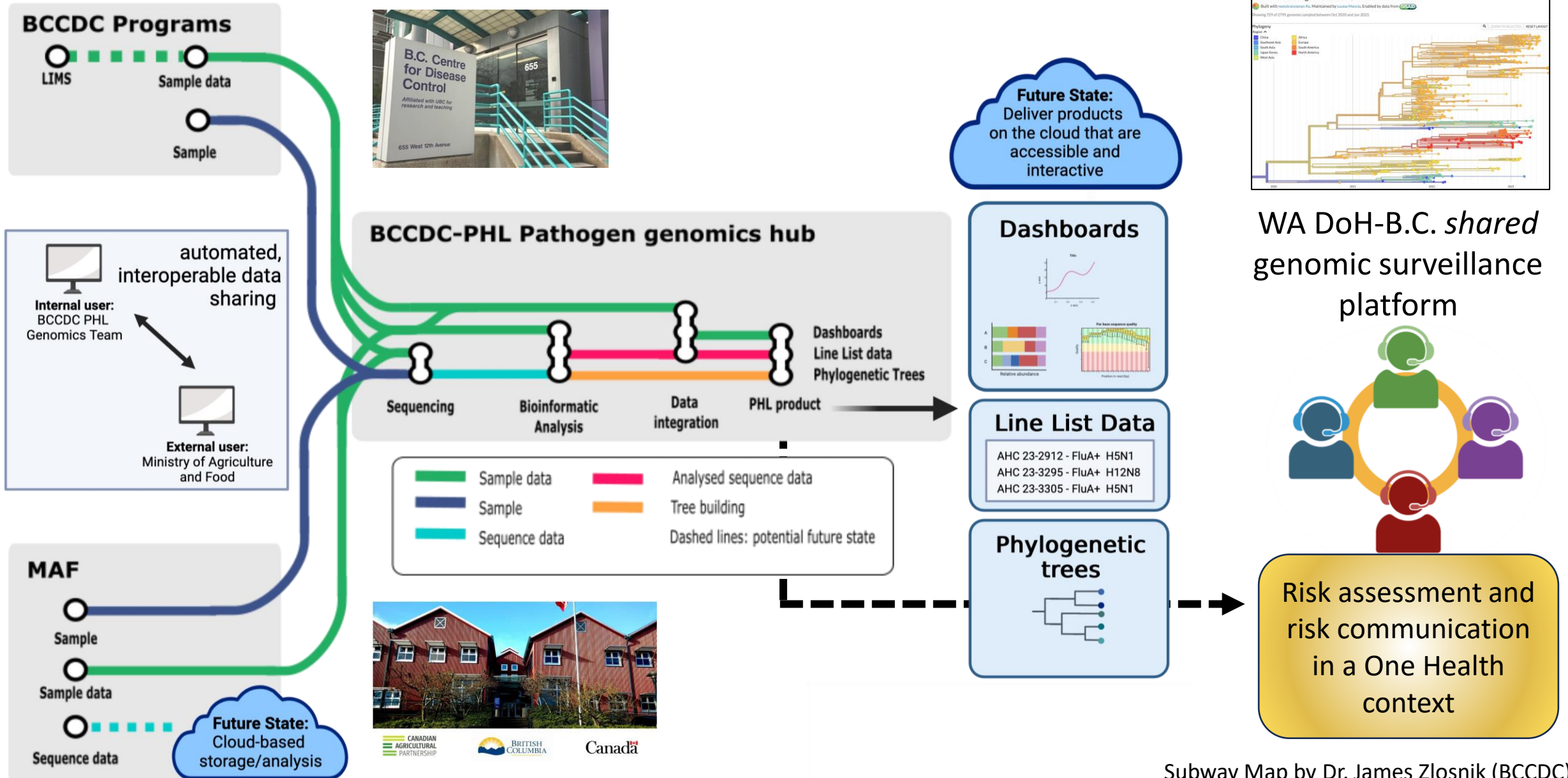


How can we use these data to better inform human/animal health risks in real-time?

Break down barriers that hinder data sharing between animal and human health sectors.



# BC One Health Strategic Plan and Genomics Program



# Acknowledgements

*So many important contributors to this work!*



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