



A WORKSHOP

NATIONAL
ACADEMIES

Sciences
Engineering
Medicine

Integrated Surveillance
Human, Animal
and Environmental Surveillance

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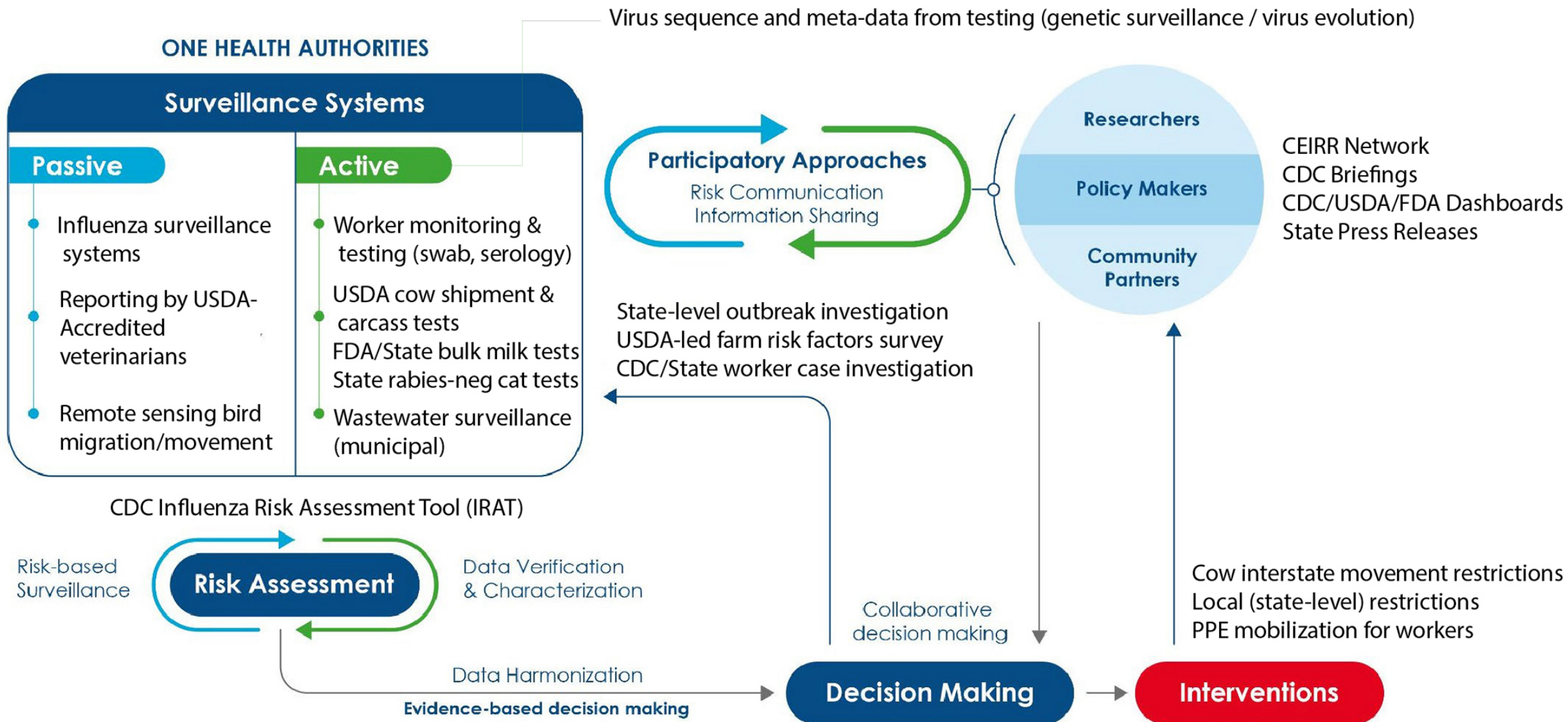


Figure adapted from the International Network for Governmental Science Advice (INGSA)-Asia Guidebook on Countering Zoonotic Spillover in the Southeast Asia Region, in collaboration with the National Academies for Sciences, Engineering and Medicine (NASEM)



Wastewater-based Detection of H5N1 Avian Influenza

Anthony William Maresso, Ph.D.

Baylor College of Medicine

Partnerships:

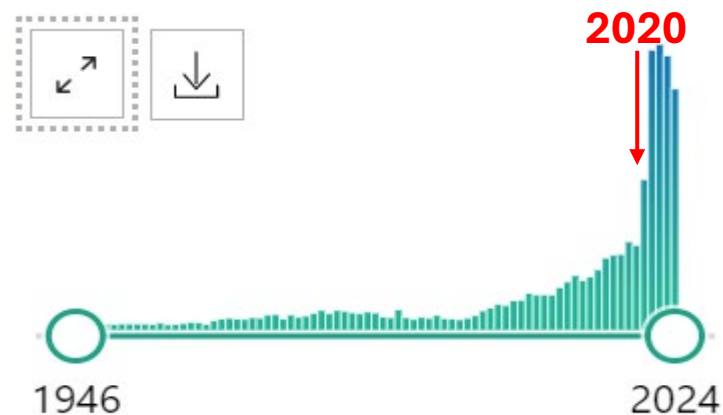


Wastewater Viral Detection 101

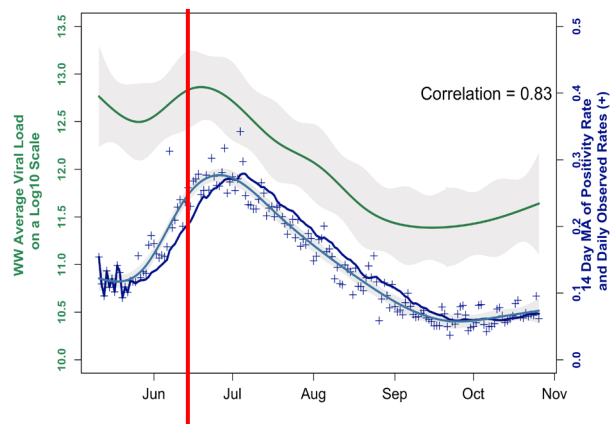
History: Polio Virus (1930-1960)



Massive Growth: Public Health Tool



Resurgence: Covid Pandemic (2020)



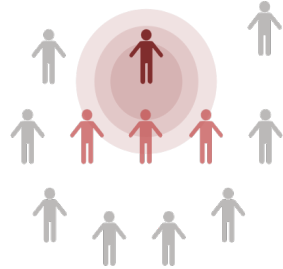
Lead Indicator

Expansion: Other Viruses

SARS-Cov2
MPXV
Flu
RSV

Sewer to Solution Cycle

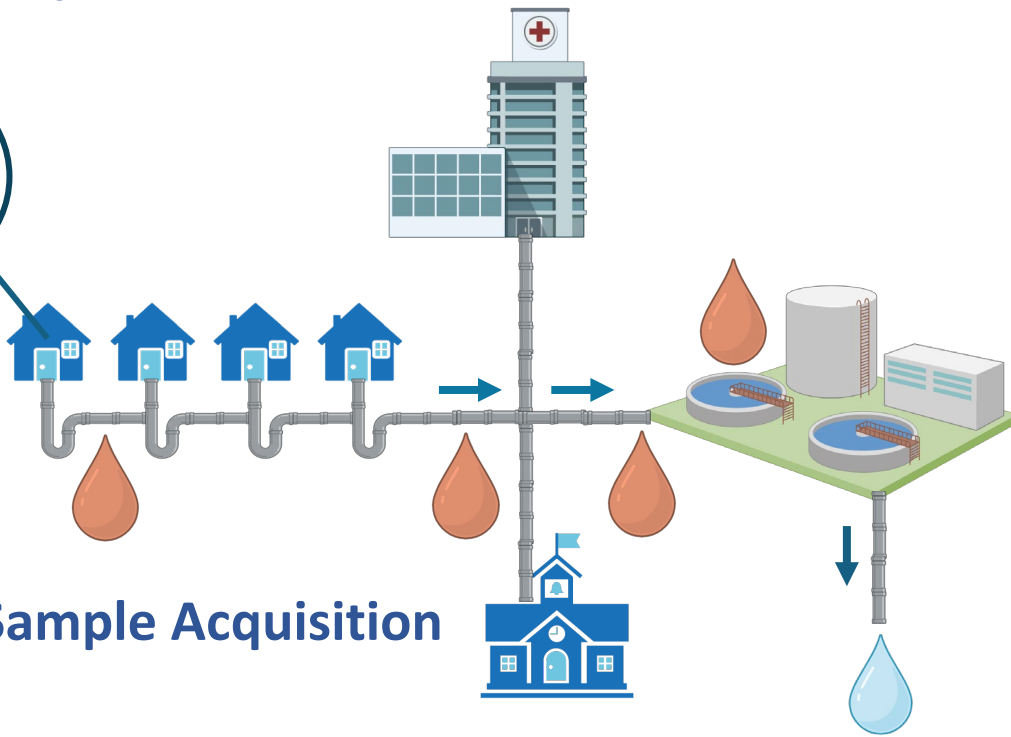
1. Public Health Situation



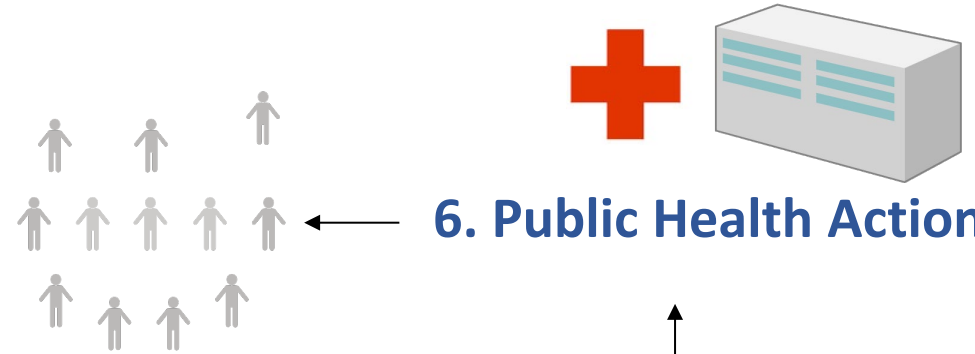
2. Sample Donation



3. Sample Acquisition

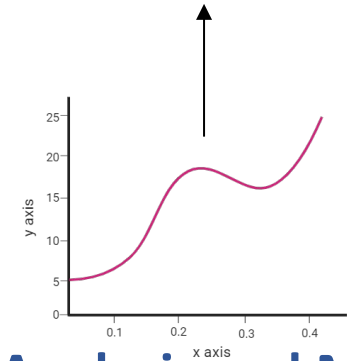


6. Public Health Action

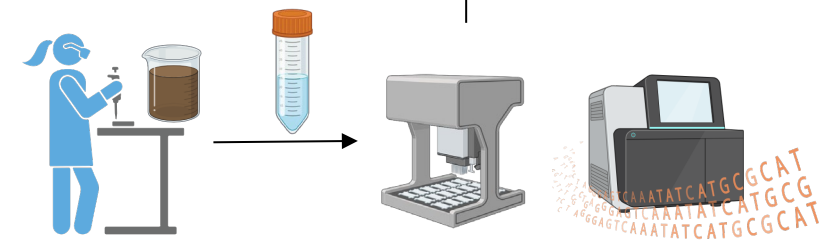


HEALTHY!

5. Data Analysis and Modeling



4. Processing and Testing



Advantage and Limitations

THE GOOD

- Population Level Data
- Cost Effective
- Universal Self-Reporting
- Comprehensive Picture
- Trend/Predictive

THE BAD

- Expectations
- Invisible Individual
- Embryonic (lot to learn)
- Uniformity (standards)
- Expertise (limited)

THE UGLY

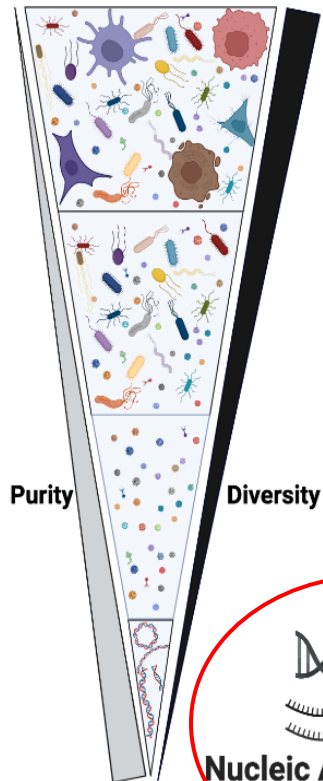
- Prevalence
- Privacy (“surveillance”)
- Politics (control)
- Pipeline (who to who)
- Policy (action?)

State-of-the-Art

Wastewater Processing

Wastewater Plant

- Centrifuge
- Filter Capture
- Bead Beat
- Centrifuge
- Collect Supernatant



Nucleic Acid Isolation

“TARGETED”

qPCR, ddPCR, rtPCR



Select Number of
Chosen Viruses

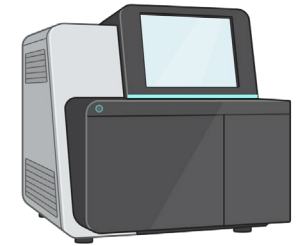
CDC Program



SARS-Cov2, MPXV, Flu, RSV

“AGNOSTIC”

Sequencing

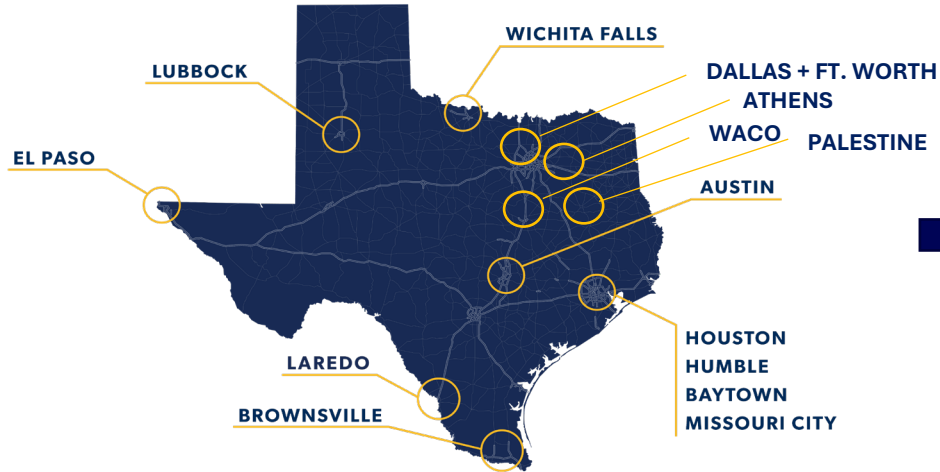


All Viruses

TexWEB

TexWEB: Texas Wastewater & Environmental Biomonitoring

Network



- **15 cities**
- **30 sites**
- **24% population**

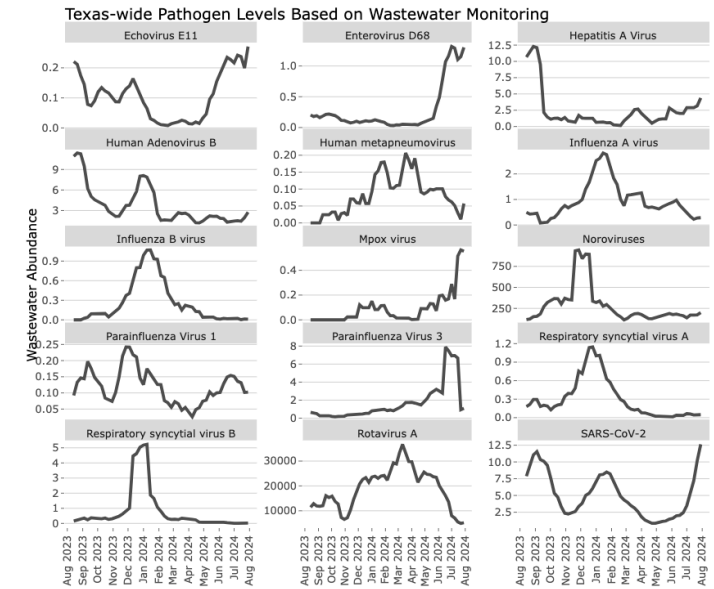
Agnostic Sequencing

- **3,153** possible viruses (1 sample)

- Detect seasonal viruses (flu, sars-cov2)

- Detect emerging viruses (measles, mpox, west nile)

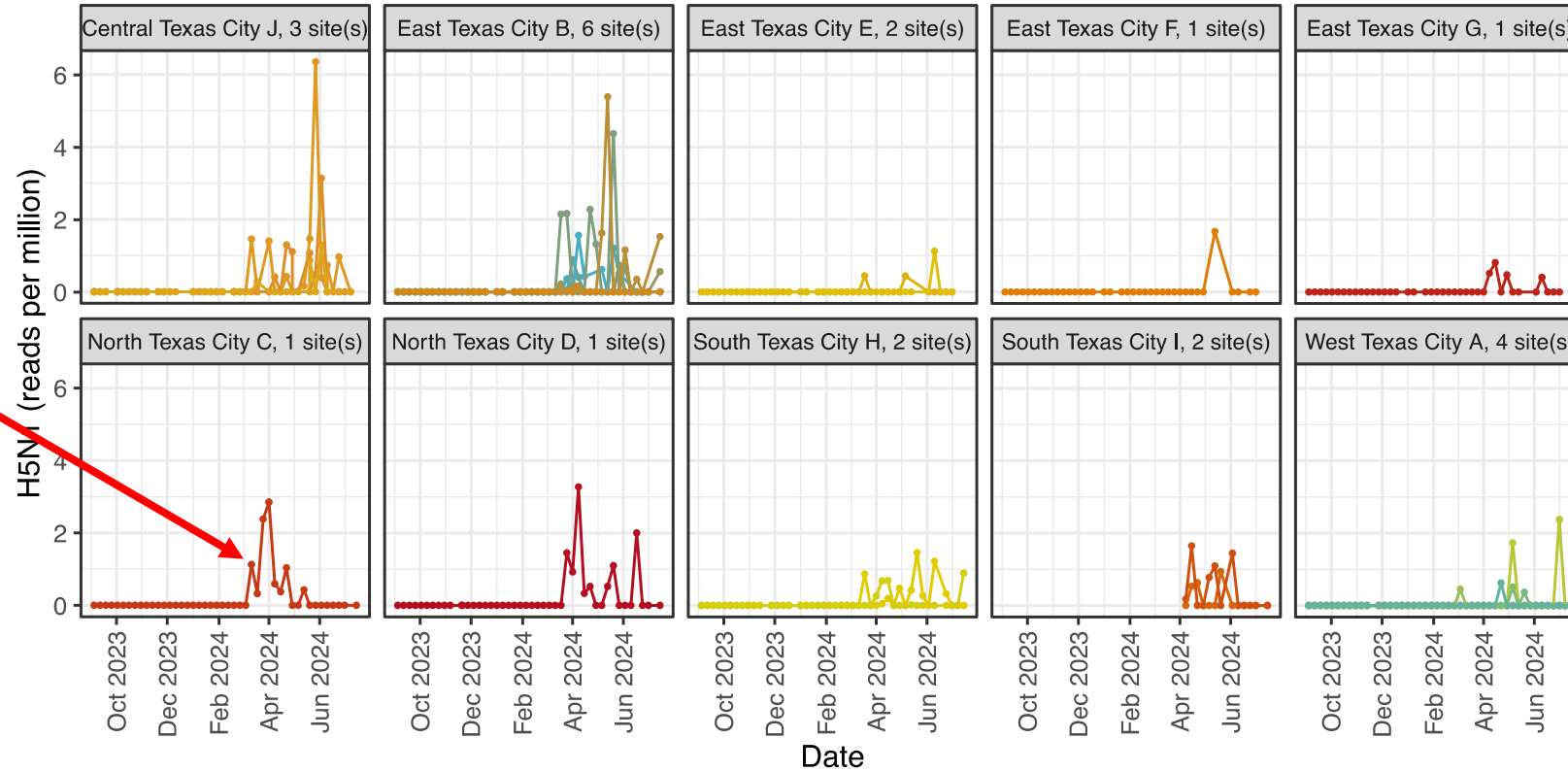
Reporting



- **Program = ~ 2.5 years**
- **Reports go to Health Dept.**

ALERT! H5N1 Detected in Wastewater Sample

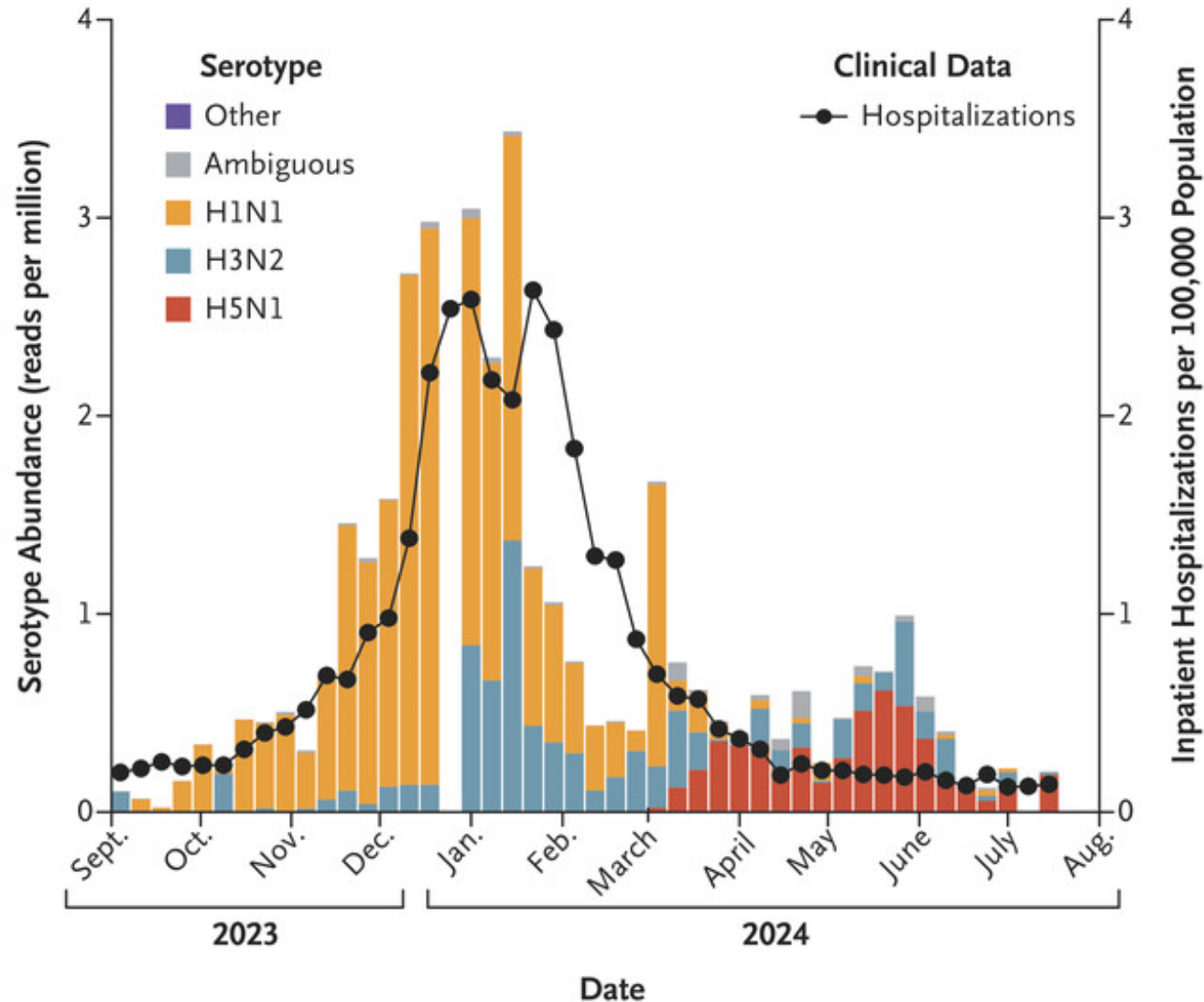
March 4th



Take Home Messages

1. **No detection** for nearly two years (**0/1337** samples)
2. About 6 weeks – **All cites** positive
3. Detection events span cities ~ **750 miles** (~ distance Washington D.C. to Chicago)

Flu Serotype Prevalence in Wastewater Sept 2023 through July 2024

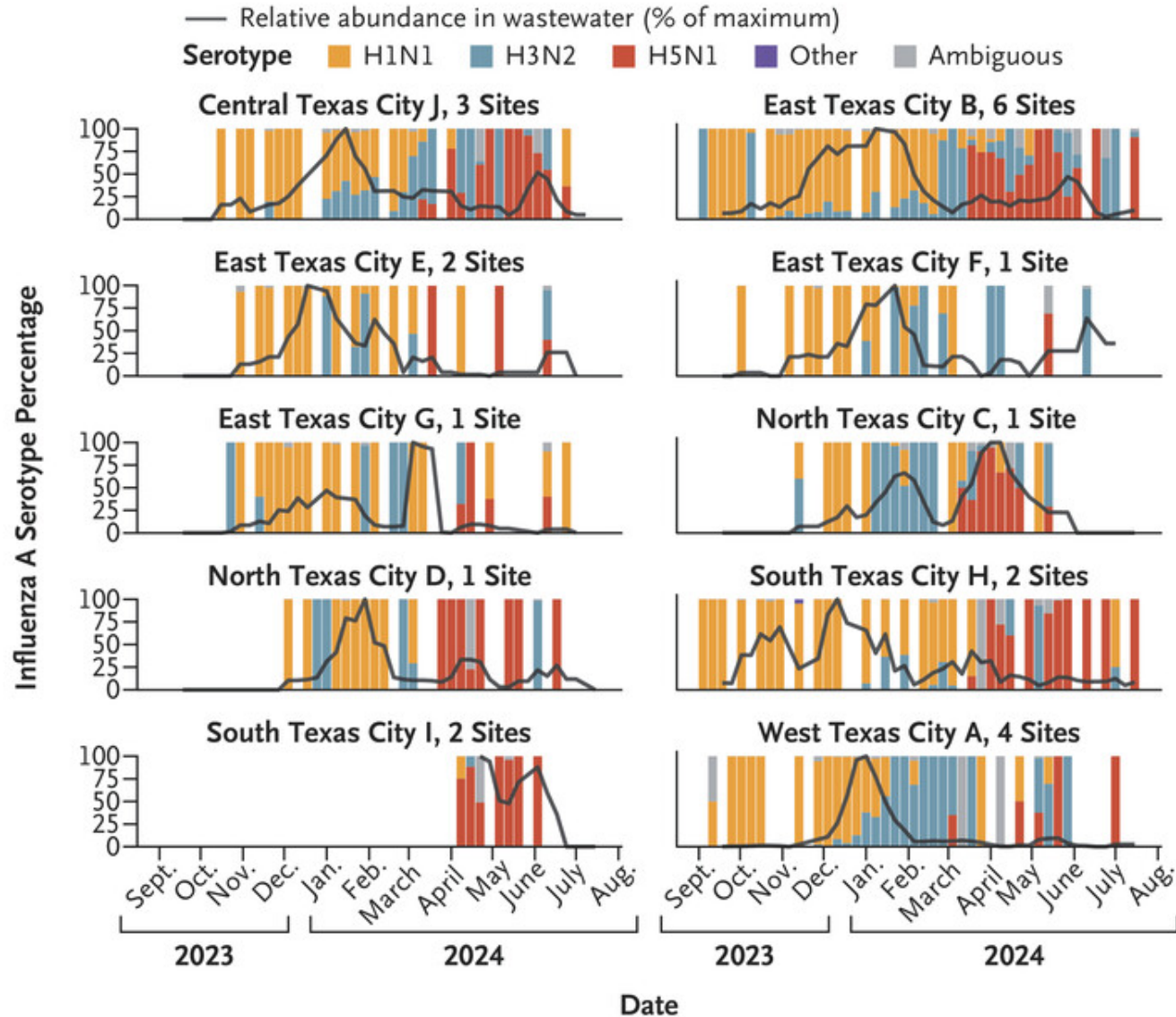


Take Home Messages

1. “Seasonal” Flu readily detected
2. “Avian” Flu – **TWO PEAKS** (April 1/May 21)
3. **No Bump** in Flu Hospitalizations

Flu Serotype Prevalence in Wastewater Sept 2023 through July 2024

Relative Abundance of Influenza A in Wastewater Samples



Take Home Messages

1. H5N1 detected in all **10 Texas cities, 22/23 sites, 25% of all samples**
2. Wide geographic distribution

Simultaneously - WasterScan Group reported PCR detection of H5

Environmental Science & Technology Letters > Vol 11/Issue 6 > Article

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Cite Share Jump to Expand

ECOTOXICOLOGY AND PUBLIC HEALTH | May 20, 2024

Detection of Hemagglutinin H5 Influenza A Virus Sequence in Municipal Wastewater Solids at Wastewater Treatment Plants with Increases in Influenza A in Spring, 2024

Marlene K. Wolfe, Dorothea Duong, Bridgette Shelden, Elana M. G. Chan, Vikram Chan-Herur, Stephen Hilton, Abigail Harvey Paulos, Xiang-Ru S. Xu, Alessandro Zulli, Bradley J. White, and Alexandria B. Boehm*

- “unusual” flu positivity in normal FluA PCR test in wastewater
- Developed H5 specific probe, retrospectively positivity 4 WWTPs
- Same time frame



Morbidity and Mortality Weekly Report
(MMWR)

Search



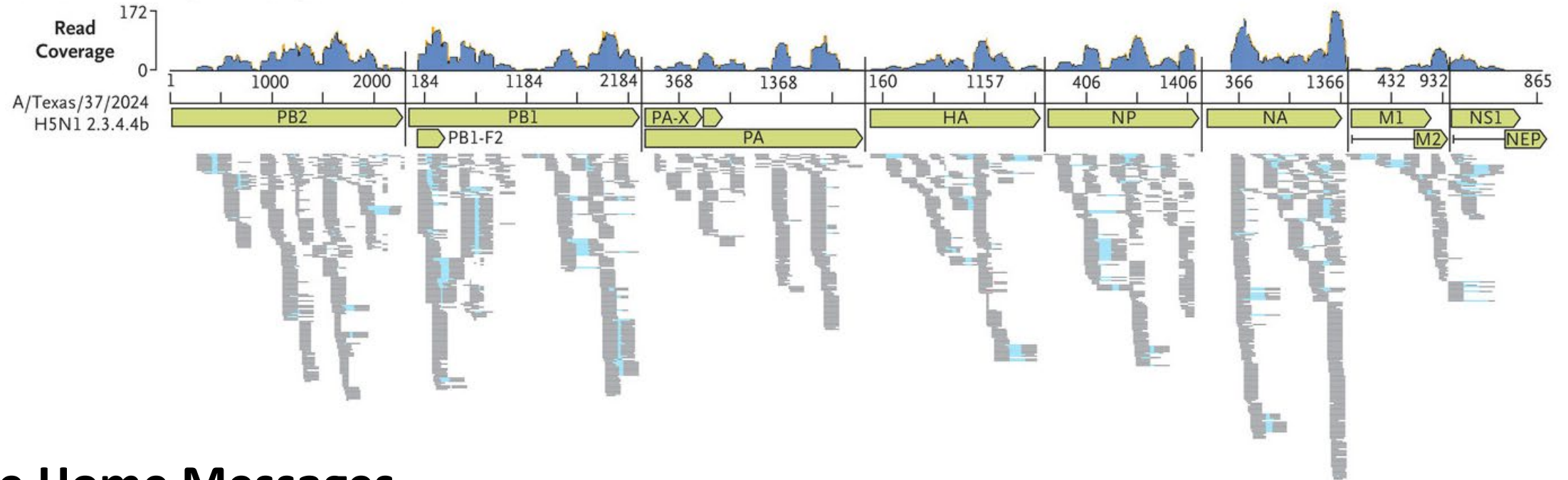
Wastewater Surveillance for Influenza A Virus and H5 Subtype Concurrent with the Highly Pathogenic Avian Influenza A(H5N1) Virus Outbreak in Cattle and Poultry and Associated Human Cases — United States, May 12–July 13, 2024

Weekly / September 19, 2024 / 73(37):804–809

- May 12–July 13, 2024.
- H5 subtype testing was conducted at 203 sites in 41 states, with **H5 detections at 24 sites in nine states.** (IE NOT JUST TEXAS WW)

H5N1 reads aligned to March 2024 Human Texas Case

Wastewater Virome Sequences Assigned to H5N1



Take Home Messages

1. Cover ~ **85%** of the ref genome
2. Reads from all **8 gene** segments

Technical Notes:

- Consensus is GISAID isolate EPI_ISL_19027114
- Read stacks might relate to probe bias

H5N1 SNPs that deviate from the Texas Case consensus

Nucleotide Position	CDS	Polymorphism Type	Change	Variant Frequency	Unique Detections (Reference Allele)	Unique Detections (Variant Allele)	Coverage	Amino Acid Change	CDS Codon Number	Codon Change	Protein Effect	Variant P-Value
1100 PB2		SNP (transition)	A->G	95.00%	0	4	40	E->G	362	GAG->GGG	Substitution	3.10E-104
1894 PB2		SNP (transition)	A->G	100.00%	0	5	36	K->E	627	AAA->GAA	Substitution	2.50E-130
1906 PB2		SNP (transversion)	A->C	100.00%	0	5	36	M->L	631	AUG->CUG	Substitution	2.50E-130
1977 PB2		SNP (transition)	G->A	100.00%	0	4	48		654	CCG->CCA	None	1.60E-173
2091 PB2		SNP (transition)	A->G	100.00%	0	1	3		692	AGA->AGG	None	7.90E-12
3502 PBI		SNP (transition)	G->A	100.00%	0	1	3	V->I	392	GUA->AUA	Substitution	7.90E-12
3612 PBI		SNP (transition)	G->A	100.00%	0	1	3		428	CAG->CAA	None	7.90E-12
4191 PBI		SNP (transition)	A->G	100.00%	0	6	47		621	CAA->CAG	None	6.30E-170
4881 PA		SNP (transversion)	C->A	100.00%	0	1	5		79	AUC->AUA	None	3.20E-19
4881 PA-X		SNP (transversion)	C->A	100.00%	0	1	5		79	AUC->AUA	None	3.20E-19
4882 PA		Substitution	GA->UG	100.00%	0	1	5		80		Truncation	3.20E-19
4882 PA-X		Substitution	GA->UG	100.00%	0	1	5		80		Truncation	3.20E-19
5068 PA		SNP (transition)	G->A	100.00%	0	2	15	E->K	142	GAA->AAA	Substitution	3.20E-53
5068 PA-X		SNP (transition)	G->A	100.00%	0	2	15	E->K	142	GAA->AAA	Substitution	3.20E-53
5299 PA		SNP (transversion)	C->A	100.00%	0	1	43	L->I	219	CUU->AUU	Substitution	1.60E-155
5299 PA-X		SNP (transversion)	C->A	100.00%	0	1	43		218	GUC->GUA	None	1.60E-155
5439 PA		SNP (transition)	A->G	17.60%	1	1	17		265	CCA->CCG	None	5.40E-09
5473 PA		SNP (transition)	C->U	15.80%	1	1	19	P->S	277	CCU->UCU	Substitution	7.70E-09
6134 PA		SNP (transition)	A->G	60.00%	1	1	10	K->R	497	AAG->AGG	Substitution	1.30E-17
6226 PA		Substitution	ACUGACCCGAG->GCCUCGGGUCA	60.00%	2	1	10	TDPR->ASGQ	528	ACU,GAC,CCG,AGG->GCC,UCG,GGU,CAG	Substitution	2.10E-19
6238 PA		SNP (transition)	C->U	42.90%	2	1	7		532	CUA->UUA	None	2.80E-10
7135 HA		SNP (transversion)	U->G	100.00%	0	1	12		93	UCU->UCG	None	4.00E-45
7387 HA		SNP (transition)	G->A	35.30%	1	1	17		177	AAG->AAA	None	1.90E-13
7519 HA		SNP (transition)	A->G	32.30%	1	1	31		221	UUA->UUG	None	4.40E-28
7657 HA		SNP (transition)	G->A	100.00%	0	6	23		267	GAG->GAA	None	6.30E-79
7723 HA		SNP (transition)	C->U	100.00%	0	6	31		289	CAC->CAU	None	5.00E-103
8094 HA		Substitution	GGGAGU->UCUGAC	22.9%->24.2%	5	1	33 -> 35		413		Truncation	1.40E-21
8170 HA		SNP (transversion)	C->A	94.10%	1	7	51		438	ACC->ACA	None	3.30E-169
9029 NP		SNP (transition)	U->C	100.00%	0	5	63		135	CAU->CAC	None	1.60E-227
9272 NP		SNP (transition)	A->G	100.00%	0	4	31		216	AGA->AGG	None	2.50E-112
9443 NP		SNP (transition)	G->A	61.90%	3	3	63		273	AAG->AAA	None	6.20E-124
9632 NP		SNP (transition)	G->A	100.00%	0	5	18		336	GCG->GCA	None	2.50E-67
9764 NP		SNP (transition)	A->G	13.00%	2	1	23		380	GAA->GAG	None	1.40E-08
10039 NP		SNP (transition)	C->U	12.70%	1	1	63	T->M	472	ACG->AUG	Substitution	9.60E-21
11065 NA		Deletion (tandem repeat)	(A)4 -> (A)3	75.60%	4	1	41		309		Frame Shift	1.70E-78
11157 NA		Substitution	UC->CA	16.70%	2	1	24	S->H	340	UCU->CAU	Substitution	1.70E-11
11160 NA		Substitution	AAUG->UAGA	16.70%	2	1	24		341		Truncation	2.70E-10
11170 NA		Substitution	AU->UG	15.4%->16.0%	2	1	25 -> 26	Y->L	344	UAU->UUG	Substitution	2.40E-11
11244 NA		SNP (transversion)	A->C	29.60%	5	1	54	S->R	369	AGC->CGC	Substitution	1.30E-46
11360 NA		SNP (transition)	C->U	39.50%	7	3	43		407	GUC->GUU	None	2.60E-50
11547 NA		SNP (transversion)	U->G	32.60%	2	4	92		470		Extension	1.50E-84
11553		SNP (transversion)	G->U	33.30%	2	4	90					6.60E-79
11556		SNP (transversion)	C->G	32.20%	2	4	87					7.80E-79
11559		SNP (transversion)	A->C	54.90%	2	3	51					3.10E-87
11565		Substitution	UAGAUUAUGA->ACUUGUCAAU	77.8%->95.7%	1	4	8 -> 23					4.50E-25
11896 MI		SNP (transversion)	A->U	42.90%	2	1	7	I->L	107	AUA->UUA	Substitution	2.80E-10
12168 MI		SNP (transition)	G->A	33.30%	3	1	12		197	GAG->GAA	None	7.80E-13
12274 MI		Substitution	CUCCUUGA->UCUAACCG	21.1%->28.6%	4	1	19 -> 21	LLE->SNR	233	CUC,CUU,GAA->UCU,AAC,CGA	Substitution	3.70E-15
12283 MI		Substitution	AA->GG	33.30%	4	1	21	N->G	236	AAU->GGU	Substitution	1.50E-21
12286 MI		Substitution	UUGCAG->CGAAAC	33.3%->58.3%	4	1	12 -> 21	LQ->RN	237	UUG,CAG->CGA,AAC	Substitution	1.50E-21

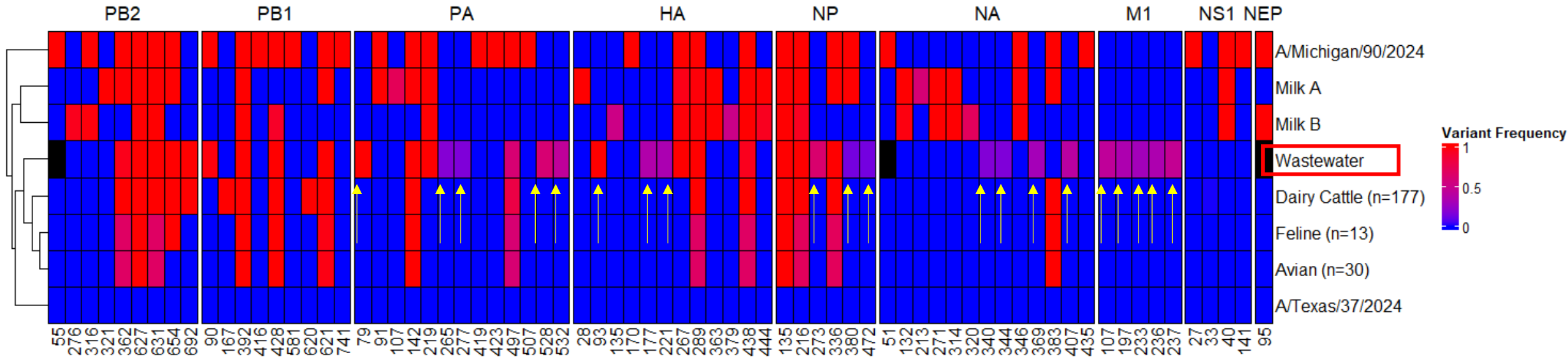
Take Home Messages

1. > 50 SNPs

2. Some SNPs are **unique** to wastewater samples

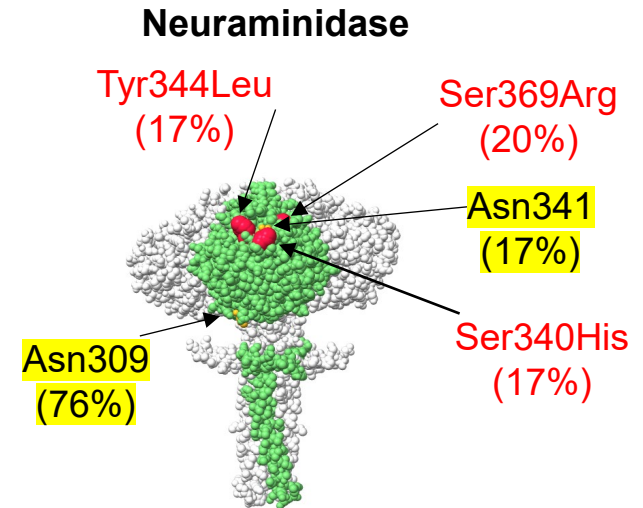
3. Inputs from cow, feline, bird, milk and **unidentified source (s)**

Wastewater Sequence is Similar but DISTINCT



Take Home Messages

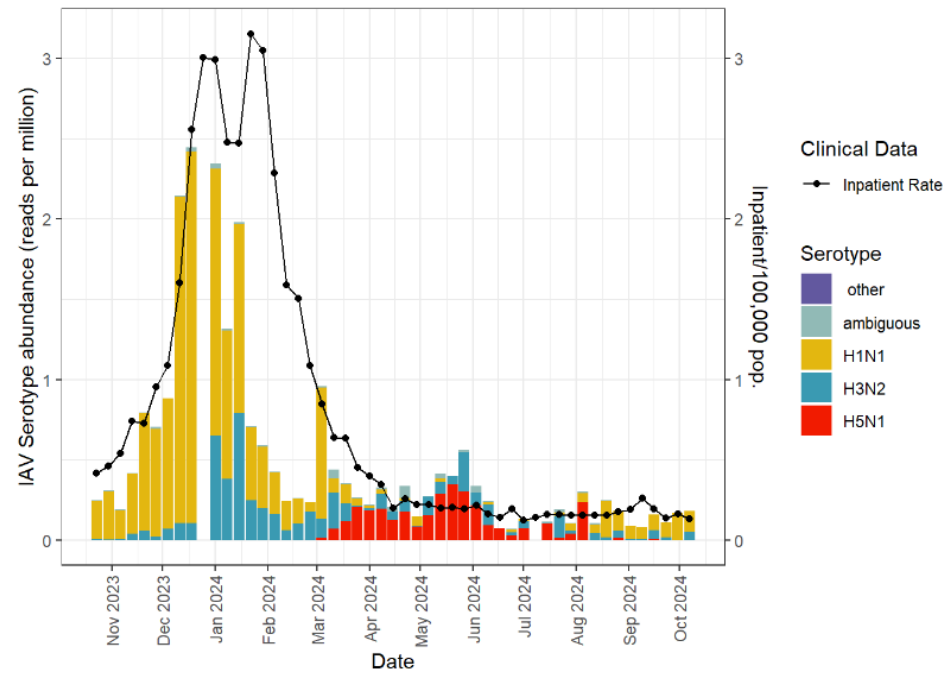
1. Wastewater has **Novel Mutations**
2. Do not know the origin of these **Mutations**
3. There are other **Cryptic Hosts** out there



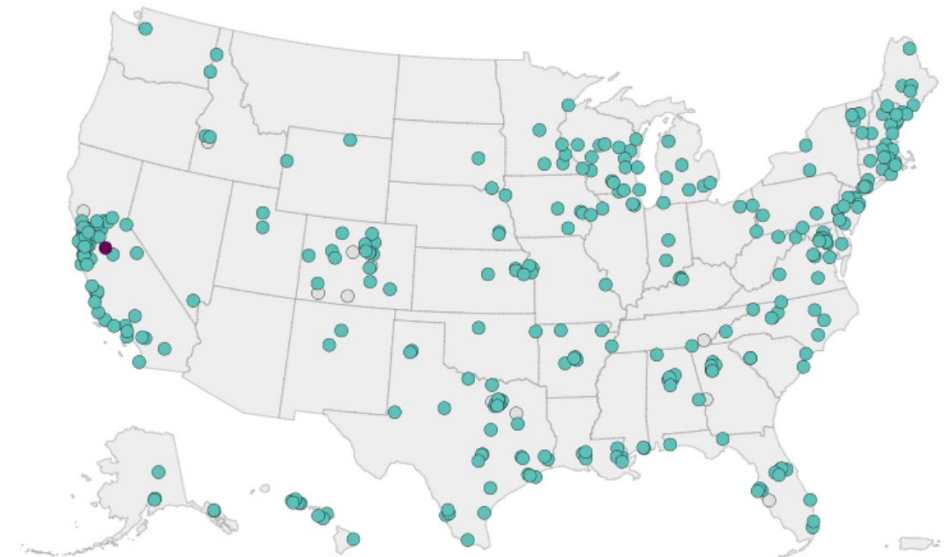
Justin Clark
James Chang

Partial Good News – Detections in WW now Low

TexWEB



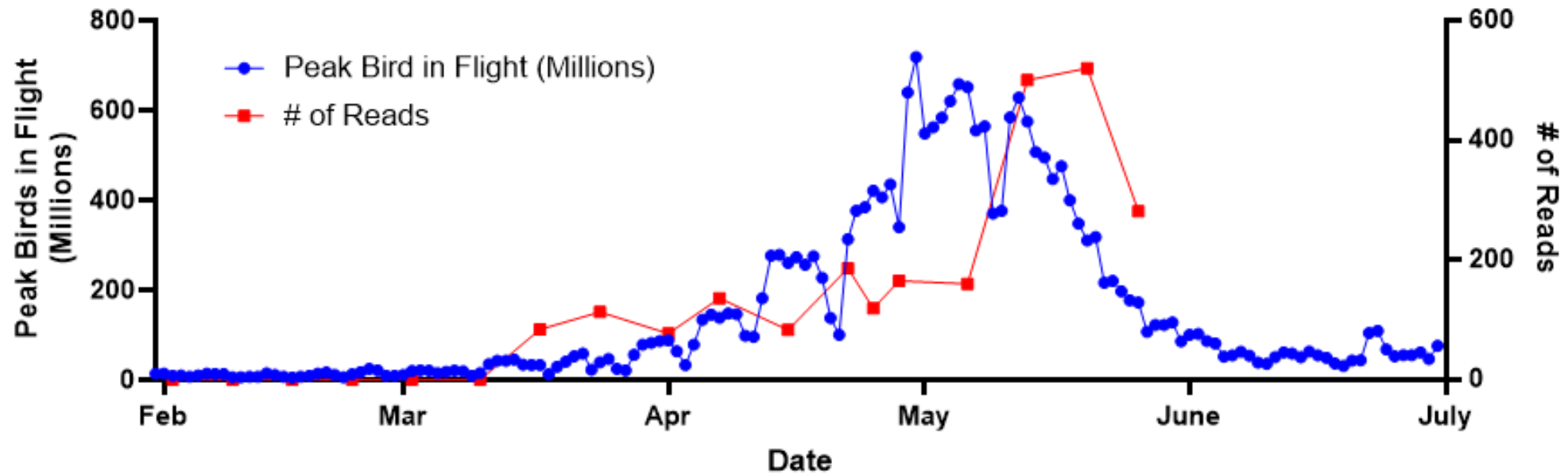
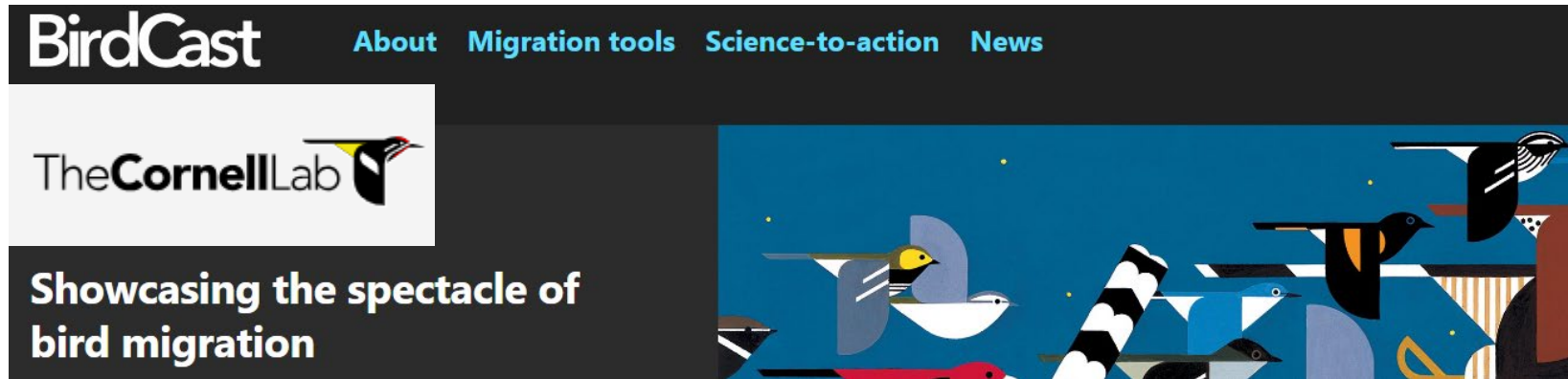
CDC



Select a detection type below to add or remove it from the map.

☒ H5 Detection ☒ No Detection ☐ No Samples in Last Week

Interesting Correlation



Major Take Home Lessons

1. H5N1 can be **readily tracked** in human wastewater

- If starts to spread in people, **confident spread can be followed**

2. Wastewater-based Agnostic Sequencing brings new capabilities

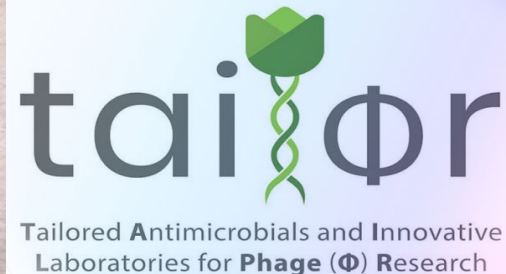
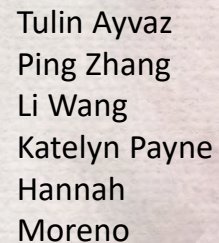
- follow **evolution and changes in near real-time**
- **Fingerprint** the source
- Correlate to **functionally meaningful** mutations/assortment in the virus
- Future of all testing (now cost effective)

3. Favor model (Texas): Migrating Birds > Cattle > Wastewater

Urge Caution: We cannot rule out human (cryptic inputs)

Anthony Maresso, Ph.D
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Justin R. Clark, Ph.D
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Keiko C. Salazar, Ph.D
Felipe Piedra, Ph.D.
John Taylor, Ph.D.
Brianna Thompson
Ellen E. Vaughan
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Yikun Xing
Jacob Zulk
Camilla Do
Dylan Chirman
Ling Qiu
Rachel Lahowetz
Alex Cervantes
Pauline J. Kim
Soufiane Bel Rhahi
Marian Fabian
Stephanie Omagari
Sam Roberts

R01AI097167
R01 AI154597
R01 NS102594
U19AI144297
U19AI116497
U19AI157981
R21AI151259
R21AI146481
VA I01-RX002595
MacDonald Foundation
Mike Hogg Foundation
Kleberg Foundation
BCM Center Funds (TAILOR)
CARES Act
Neilson Foundation
87th Legislature TEXAS



A Practical One Health Strategy to Detect Pre-Pandemic Zoonotic Respiratory Virus Threats

utmb Health

Working together to work wonders.



Gregory C. Gray, MD, MPH, FIDSA

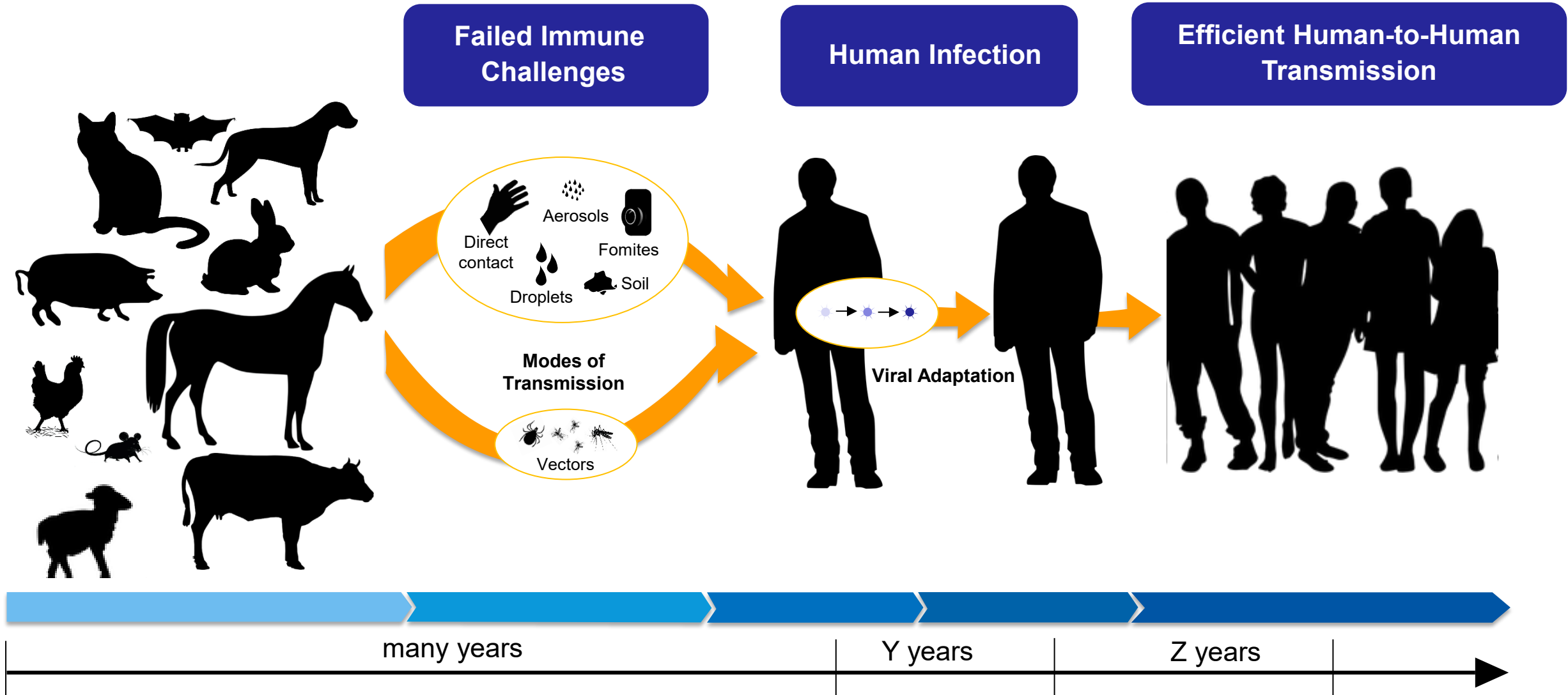
University of Texas Medical Branch (UTMB)

Robert E. Shope, MD, Professor in Infectious Disease Epidemiology, Departments of Internal Medicine (Infectious Diseases), Microbiology and Immunology, and Global Health & Emerging Disease

Photo by: Jose Carlos Fajardo/Bay Area News Group

A Model for Zoonotic Pathogen Genesis

Viral evolutionary studies suggest this progression may take many years



Chances of finding COVID-virus ancestor ‘almost nil’, say virologists

Genome analysis finds SARS-CoV-2 and bat coronaviruses shared an ancestor just a few years ago, but extensive recombination has muddled the picture.

Evolutionary clock ticks
slowly

...More than a dozen viruses closely related to SARS-CoV-2 have been isolated from bats and pangolins so far. ...SARS-CoV-2's closest known relatives are a bat virus found in Laos called BANAL-52, whose genome is 96.8% identical to that of SARS-CoV-2, and a virus called RaTG13, found in Yunnan, southern China, which is 96.1% identical¹. The 3–4% difference between their genomes and that of SARS-CoV-2 suggests that **there has been about 40–70 years of evolution since these viruses shared a common ancestor.**

Megafarms

105,000 SOWS STACKED SIX STORIES HIGH

THE MEGA FARM IN CHINA HAS 21 BUILDINGS WITH 5,000 SOWS EACH, FARROW TO FINISH.

By [Betsy Freese](#)

4/28/2021



Listen to article 3 minutes



Construction of the Muyuan Meat and Food Industry complex in China, with 105,000 sows in 21 six-story buildings, is progressing steadily, reports Michael Ellermann, a swine consultant with Danish company Aspire Agritech Consulting. Ellermann is based in China, where he works with clients on training, farm management, and new farm development.

The site covers 450 acres, with another 500 acres in a logistics park with railway and highway ports. The feed mill on the site will produce 720,000 tons of feed annually.



China's mega-farm... for 100,000 cows: World's biggest dairy being built to supply Russian demand after Moscow boycotted EU exports

- Farm in Mudanjiang City will have 60,000 more cows than current biggest
- Russia wants milk as it's boycotting EU countries' milk and dairy exports
- It will be 50 times bigger than the UK's biggest, which has 2,000 cows

By [DAILY MAIL REPORTER](#)

PUBLISHED: 21:05 EDT, 10 July 2015 | **UPDATED:** 21:30 EDT, 10 July 2015



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China is building the biggest dairy farm in the world with 100,000 cows to supply Russia.

The enormous farm in Mudanjiang City will have 60,000 more cows than the current biggest farm, also in China.

Russia wants the milk as it is boycotting EU countries' milk and dairy exports after Brussels imposed economic sanctions after the Ukraine crisis.

Problems

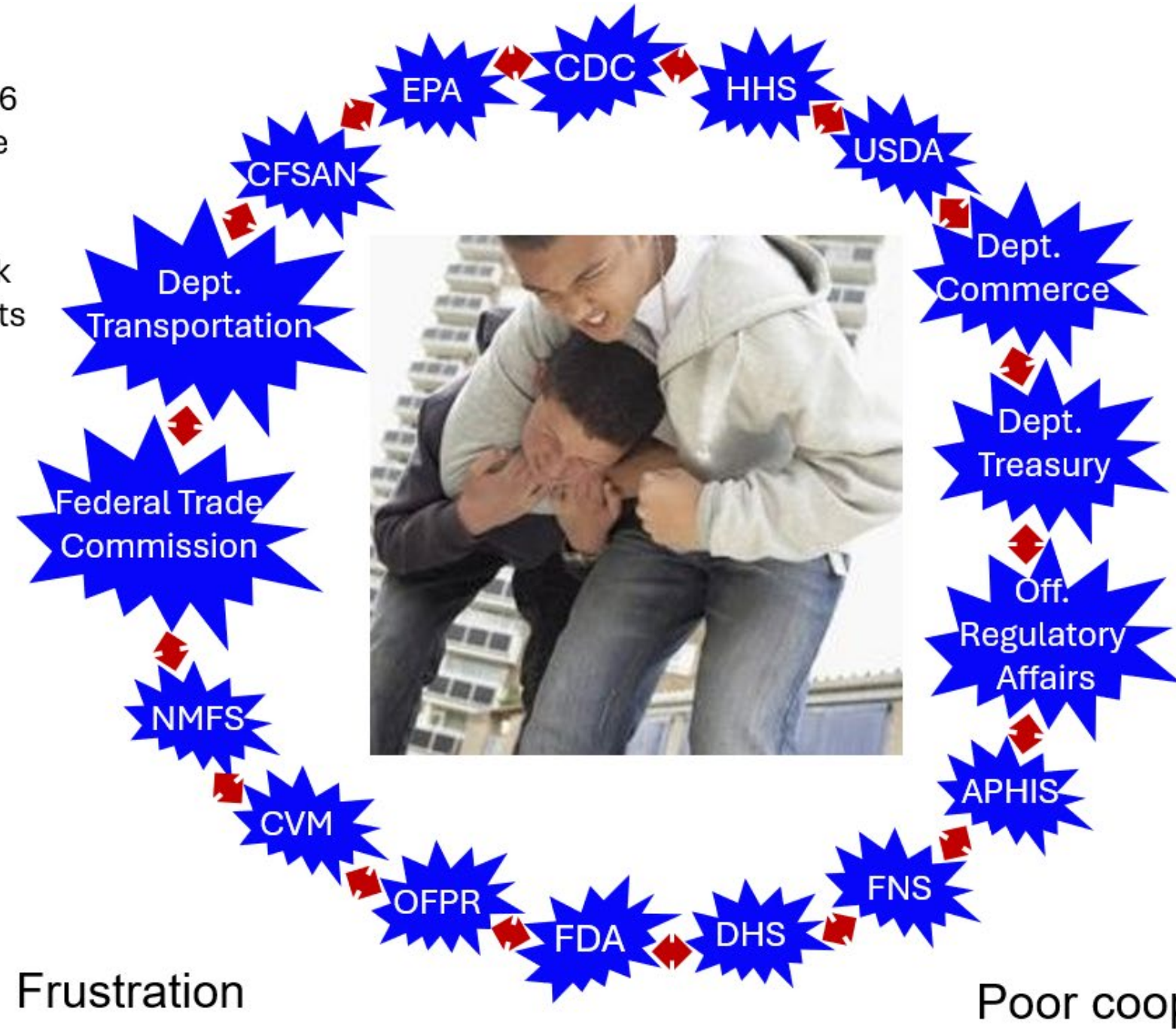
- Little trust between livestock farms and government institutions
- No effective surveillance for novel pathogen emergence on livestock farms
- While we have theoretically have time to detect and respond to spillover threats, in truth we currently have a “whack a mole” mentality that is very ineffective



Image taken from
<https://www.youtube.com/watch?v=VoP1E9J4jpg&feature=youtu.be>



Food Security -It has been estimated that 16 federal agencies share food safety responsibilities and they don't always work well together. This hurts consumers and food industries



Pigs, pathogens, and public health



On Nov 8, 2017, Presidents Donald Trump and Xi Jinping announced a US\$8 billion trade agreement, part of which will further increase US pork and animal feed exports to China. Given this expansion of global pork trade, now seems an appropriate time to consider emerging pathogen threats that accompany modern pork production, particularly in China. China has been implicated as the site of origin of the 1957 and 1968 influenza pandemics¹ and is thought to be an epicentre for future novel influenza virus emergence.² With increasingly dense populations of pigs, poultry, and people, and often weak farm and animal market biosecurity,³ it is little wonder that novel influenza A viruses, resulting in increased morbidity and mortality among both livestock and human populations, have emerged in China. China is also recognised as the site of emergent novel pig-only pathogens, such as the strains of porcine reproductive and respiratory syndrome virus (2006)⁴ and porcine epidemic diarrhoea virus (2014),⁵ which have resulted in hundreds of millions of dollars of agricultural losses in China and the USA.

China's 2017 population of 1.41 billion is growing at an annual rate of about 0.45%, translating to an annual increase of more than 6 million additional citizens.⁶ Even with moderate growth, China's pork production and consumption—now over half of the world's—will be enormous. Pork is so important to China that in 2007 it established a national pork reserve.⁷ While China's Ministry of Agriculture currently seeks to transform domestic pork production by constructing large US-style industrial animal facilities, it has increasingly turned to cheap, nutritious, and safe imported pork. Indeed, a Chinese holding company is now the largest US pork producer but the center of US pork production remains in the state of Iowa, which now has more than 14 000 animal feeding operations (AFOs). With growth of these operations almost unchecked, new Iowa AFO permits are projected to exceed 500 this year, while two huge pork processing facilities are under construction to meet demand driven by China's imports, with trade being facilitated by Iowa's former five-term governor, Terry Branstad, now US Ambassador to China.

Studies in the USA and China have documented the effect of industrial swine production and animal

transport on the number of novel swine influenza A viruses in swine herds. Most recently, these novel or variant, swine-reservoir influenza A viruses have been increasingly infecting swine workers, most likely their family members and individuals without previous pig exposures attending agricultural fairs.⁸ Studies have also documented introduction of human-reservoir influenza A viruses into pig herds, increasing the variety of novel viruses found in pigs from which yet more novel viruses could arise. Also, we have found that living in close proximity to US swine farms could increase the risk of an influenza-like illness possibly from influenza viruses that are amplified in pigs.⁹

An intensive study of swine and swine workers has documented strong evidence of influenza A virus mixing, likely reassorting, and cross-species infections on Chinese farms.¹⁰ This study also documented sparse use of personal protective gear among workers, poor biosecurity (frequent mixing of animal species, figure), and essentially no monitoring for novel virus emergence. Far from adequate in the USA, there is now an opportunity—indeed, an imperative need—for bilateral



Figure: Mixing of poultry and pigs in Chinese pig farms
In Chinese pig farms it is not uncommon to see other species of animals (poultry, passerine birds, dogs, or rodents) freely mixing with pigs. These other animals can introduce new strains of influenza A virus to the pigs, increasing the variety of influenza A viruses circulating in farms. Continual introduction of new piglets in large farms maintains virus replication and supports the mixing of viral genes, which could generate novel progeny viruses. These progeny viruses could cause epidemics in pigs or human beings. Photo credit: Greg Gray, China; November, 2016.

To Succeed, One Health Must Win Animal Agriculture's Stronger Collaboration

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The One Health approach has received widespread international endorsements from professional, academic, and governmental organizations as the way forward in tackling complex interdisciplinary problems, such as emerging zoonotic diseases, antimicrobial resistance, and food safety. Yet conspicuously absent from US One Health training or research activities are the animal agricultural industries. Their absence is likely due to multiple factors, including the lack of appreciation for their potential problem-solving roles, as well as the industries' business-oriented fears that such engagement could cause them to suffer economic damage. As demands on the swine, poultry, egg, beef, and dairy production industries are closely linked to the above-mentioned complex problems, we must find new, nonthreatening ways to better engage and win animal agriculture's collaboration into One Health training and research partnerships for successful health problem solving. Without animal agricultural industries' improved cooperation, One Health's efforts to control these complex problems are not likely to succeed.

Keywords. biosecurity; One Health; zoonotic diseases; antimicrobial resistance, animal agriculture.

The One Health approach [1], a strategy where professionals from multiple and often disparate disciplines work together to solve complex health problems, has gained much notoriety in recent years. It has been endorsed by many professional organizations [2, 3], academia [4], governments [5, 6], and multinational institutions [7] as the best way forward in responding to the very complex issues, such as antimicrobial resistance (AMR) [3], emerging pathogens [8], and food safety [9]. Each of these complex problems requires and has the enthusiastic engagement of professionals from human health, animal health, and environmental health [10]. However, despite improvements in agricultural industries' engagement in One Health policy in recent years, applied consortia that target these complex problems through training and research frequently lack the enthusiastic participation of modern animal agriculture industries, without which they are not likely to succeed. For instance, how can we know which AMR genes or emerging pathogens are circulating in modern farms and design effective interventions to counteract them unless we aggressively surveil and share the data with collaborating problem-solving stakeholders? Despite observations that large pig farms were involved in the evolution of the 2009 pandemic influenza A virus [11, 12], routine

surveillance data for the next pandemic influenza A virus circulating among pigs are remarkably sparse [13]. Unfortunately, AMR and emerging pathogen research efforts in academia are often not inclusive of animal agriculture industries. This omission could be due to a lack of established and ongoing collaborations—and, thus, professional trust—across these sectors, as well as other practical challenges, especially with data sharing. Although there have been great strides in addressing AMR in animal agriculture, some industries may still not wish to engage in AMR and emerging pathogens research, as it is reasonable to perceive that the work could be a threat to industries' profit generation if not closely managed. Unfortunately, the limited surveillance that is openly reported is most often released in response to overt animal illnesses or to concerns raised by others about human health issues, such as food contamination [14]. While proactive assessments and interventions for AMR and emerging pathogens may be occurring, only limited AMR and emerging pathogens surveillance data that animal agriculture industries collect are released for broad-scale analyses, unlike the commitment seen to open data sharing in other realms.

While international and national organizations are engaging animal agriculture in One Health discussions, animal industries' sparse engagement in One Health (ie, collaborative) research becomes very apparent when attending national or international One Health conferences, reading the One Health scientific literature, or discussing human health biosecurity. Industries are not commonly invited to the table and, therefore, may be blamed in absentia, often inappropriately, for abetting the complex problems. Animal agriculture's absence is not from a lack of awareness, as evidenced by separate,

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One Health Studies for Novel Respiratory Viruses on Farms

Avian Influenza A(H5N1) Virus among Dairy Cattle, Texas, USA

Judith U. Oguzie,¹ Lyudmyla V. Marushchak,¹ Ismaila Shittu, John A. Lednicky, Aaron L. Miller, Haiping Hao, Martha I. Nelson, Gregory C. Gray

During March and April 2024, we studied dairy cattle specimens from a single farm in Texas, USA, using multiple molecular, cell culture, and next-generation sequencing pathogen detection techniques. Here, we report evidence that highly pathogenic avian influenza A(H5N1) virus strains of clade 2.3.4.4b were the sole cause of this epizootic.

had transient respiratory and gastrointestinal signs (5). Veterinary diagnostic laboratory results were largely unremarkable except for rumors among cattle veterinarians of possible influenza A virus detection among cattle and conjunctivitis among dairy farm workers. The University of Texas Medical Branch (UTMB) research team offered diagnostic support owing to the team's novel pathogen

- 7 (29%) of 24 nasal swab samples from sick cows had molecular evidence of highly-pathogenic H5N1 influenza A (HPAIV).
- Sick cattle had no evidence of co-infecting viruses such as influenza D, adenoviruses, coronaviruses, enteroviruses, paramyxoviruses, or pneumoviruses

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A One Health Investigation into H5N1 Avian Influenza Virus Epizootics on Two Dairy Farms

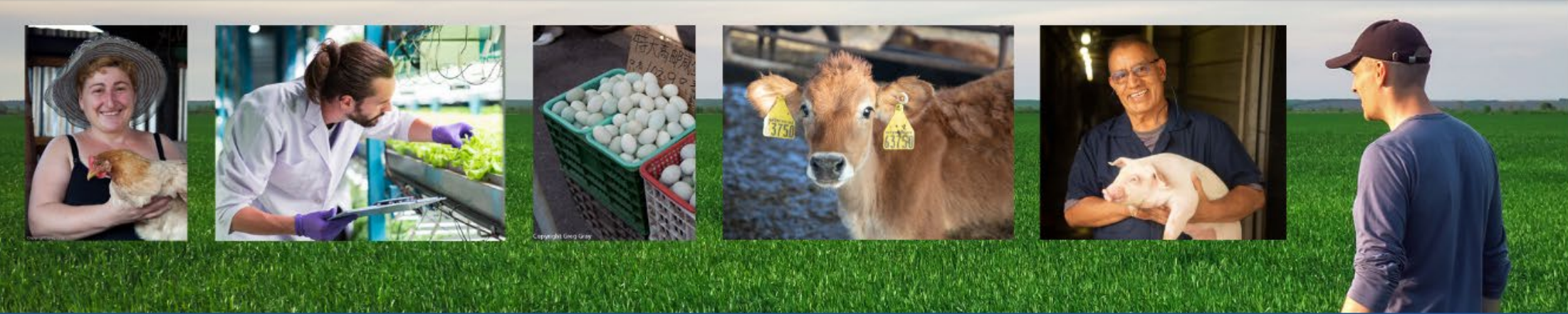
Ismaila Shittu, Diego Silva, Judith U. Oguzie, Lyudmyla V. Marushchak, Gene G. Olinger, John A. Lednicky, Claudia M. Trujillo-Vargas, Nicholas E. Schneider, Haiping Hao, Gregory C. Gray

doi: <https://doi.org/10.1101/2024.07.27.24310982>

- During April 3rd & 4th 2024, we enrolled two Texas dairy farms (Farm A & B) in our USDA-sponsored One Health study....The farms were recovering from the cattle HPAIV H5N1 epizootic
- We detected H5N1 HPAIV in 64% (9/14) of milk specimens, and 2.6% (1/39) of cattle nasal swab specimens.
- 14.3% (2/14) of the farm workers (all on farm A) who donated sera were recently symptomatic and had elevated neutralizing antibodies against a related H5N1 strain.

NGS Sequencing Results from Recent Sick Beef Cattle Nasal Swabs Collected from Indiana, Kentucky, and Mexico, Feb to Aug in 2024

Virus Identified	Estimated percent of the viral genome assembled	Farm Where Detected
Bovine rhinitis A virus	100%	Mexico farms; Kentucky farm 1, 2, 3; Indiana farm 1
Bovine coronavirus	99.90%	Mexico farms; Kentucky farm 1, 2, 3; Indiana farm 1
Bovine astrovirus	98.80%	Mexico farms
Bovine <u>nidovirus</u>	98.40%	Mexico farms; Indiana farm 1
Enterovirus E virus	97.30%	Mexico farms; Kentucky farm 2
<u>Nodaviridae spp</u>	96.30%	Mexico farms
Bovine <u>picobirnavirus</u>	92%	Mexico farms
<u>Praha dicistro-like virus 2</u>	90.90%	Kentucky farm 1
Bovine rotavirus	88.90%	Kentucky farm 1; Indiana farm 1
Bovine rhinitis B virus	82.90%	Mexico farms; Indiana farm 1
Bovine respiratory syncytial virus	80.60%	Mexico farms
<u>Flumine dicistrovirus 40</u>	72.70%	Kentucky farm 1
<u>Rodent coronavirus/Lucheng Rn rat coronavirus from bioaerosol and nasal swabs of cows</u>	33.00%	Mexico farms



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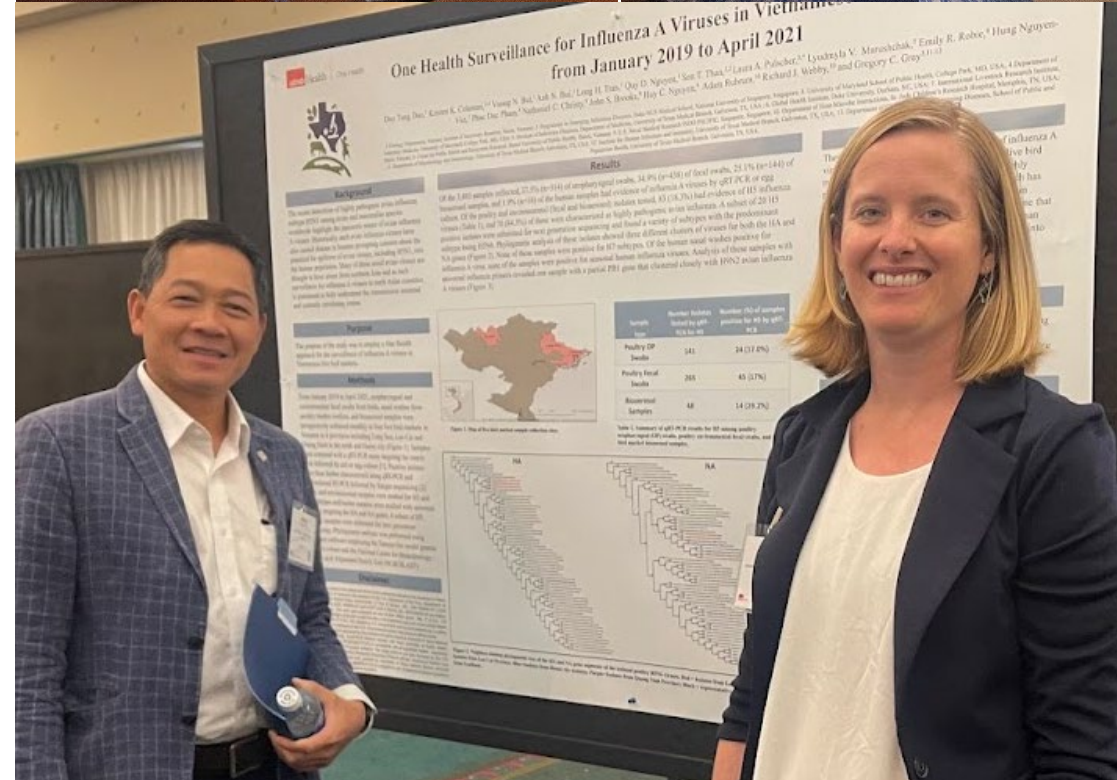
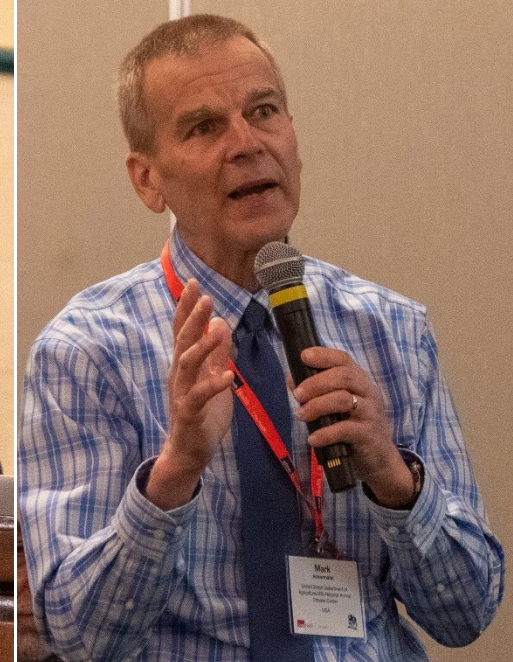


Elsevier *One Health Journal*

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- 124 science attendees from 10 countries
- 30 oral presentations from research leaders in agriculture, academia, and government
- 44 poster presentations with 16 awards (10 best abstract, 6 best poster)
- Special issue of the One Health journal – all abstracts and ~10 manuscripts from the best presentations



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Leaders call for scale-up in implementing One Health approach

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ONE HEALTH

OPERATIONAL FRAMEWORK FOR STRENGTHENING
HUMAN, ANIMAL, AND ENVIRONMENTAL PUBLIC HEALTH
SYSTEMS AT THEIR INTERFACE



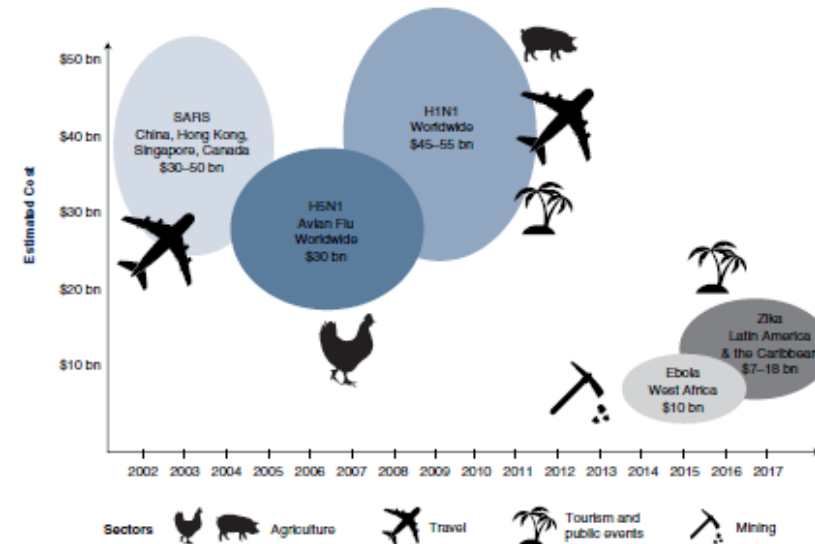
CHAPTER

2

Value of Investing in One Health

Given the high cost of emerging diseases as well as the persistent burden of endemic diseases (see Figure 2.1 and Table 2.1), One Health should be considered to assist client countries in strengthening their ability to address known and potential disease threats at the human-animal-environment interface. For a One Health approach to be warranted, it must provide added value. Fundamentally, strong sectoral health systems (e.g., human health, animal health, environmental health) must be in place—or existing systems strengthened—to support effective coordination and collaboration. Relevant metrics for value generation depend on the goal of an investment or client country, but in general, One Health offers synergies among these sectoral systems, providing expanded capacity and effectiveness in prevention of damages and/or control of disease, efficiency, and ultimately financial savings.

Figure 2.1: Examples of economic impacts of disease outbreaks (see also Table 2.1); icons represent examples of highly-affected sectors.



Figures are estimates and are presented as relative size. Based upon BioEris, World Bank, and UNDP data. Chart updated by EcoHealth Alliance.

We Have Multiple Government Agencies Calling for One Health Research Approaches



US Government Agency	One Health Program/web page
CDC	https://www.cdc.gov/one-health/about/
DoD	https://wrair.health.mil/Biomedical-Research/Center-for-Infectious-Disease-Research/One-Health/
Department of Homeland Security	https://www.dhs.gov/nbic
FDA	https://www.fda.gov/animal-veterinary/animal-health-literacy/one-health-its-all-us
HHS	https://www.hhs.gov/sites/default/files/day2-05-behnavesh.pdf
Institute of Medicine	https://www.ncbi.nlm.nih.gov/books/NBK100665/
NASA	https://appliedsciences.nasa.gov/sites/default/files/NASA_OneHealth.pdf
National Park Service	https://www.nps.gov/orgs/1632/index.htm
NOAA	https://cpo.noaa.gov/noaa-one-health/
The White House	https://www.whitehouse.gov/wp-content/uploads/2022/10/National-Biodefense-Strategy-and-Implementation-Plan-Final.pdf
USAID	https://www.usaid.gov/biodiversity/stories/human-health-environment
US Department of State	https://www.state.gov/dipnote-u-s-department-of-state-official-blog/science-speaks-one-health/
US EPA	https://www.epa.gov/one-health
USDS	https://www.usgs.gov/mission-areas/ecosystems/news/usgs-one-health-approach-infectious-diseases-wildlife-and
USDA APHIS	https://www.aphis.usda.gov/one-health
USDA	https://www.usda.gov/topics/animals/one-health
US Fish & Wild-Life Service	https://www.fws.gov/story/2023-06/international-affairs-and-one-health



The Problem in a Nutshell

- No one discipline is trained to engage such complex infectious disease problems
- No one agency or organization can control such complex infectious disease problems

Centers of Excellence for Influenza Research and Response (CEIRR)

A NIAID funded international research network created to study influenza and combat influenza outbreaks.



About CEIRR

The Centers of Excellence for Influenza Research and Response (CEIRR) Network is a multidisciplinary and collaborative research network funded by the [National Institute of Allergy and Infectious Diseases \(NIAID\)](#) to study the natural history, transmission, and pathogenesis of influenza and provide an international research infrastructure to address influenza outbreaks.

CEIRR Centers conduct studies to study key influenza research areas and provide pandemic response. CEIRR projects aim to better understand the immune response to influenza vaccination and infection, and identify immunological factors that contribute to disease severity. Surveillance projects investigate how influenza viruses infect, evolve, and transmit in both human and animal infections. Although CEIRR is primarily focused on influenza, the Network also studies severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, as well as other emerging viruses of pandemic potential.

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CRIPT & Penn-CEIRR researchers mimicked pasteurization conditions to investigate the impacts of temperature on influenza A(H5N1) virus stability in different types of dairy milk. Read a summary here: <https://ow.ly/IKyJ50Sj1ct>

Effects of Temperature and Composition of Dairy Milk on Influenza A Virus Stability

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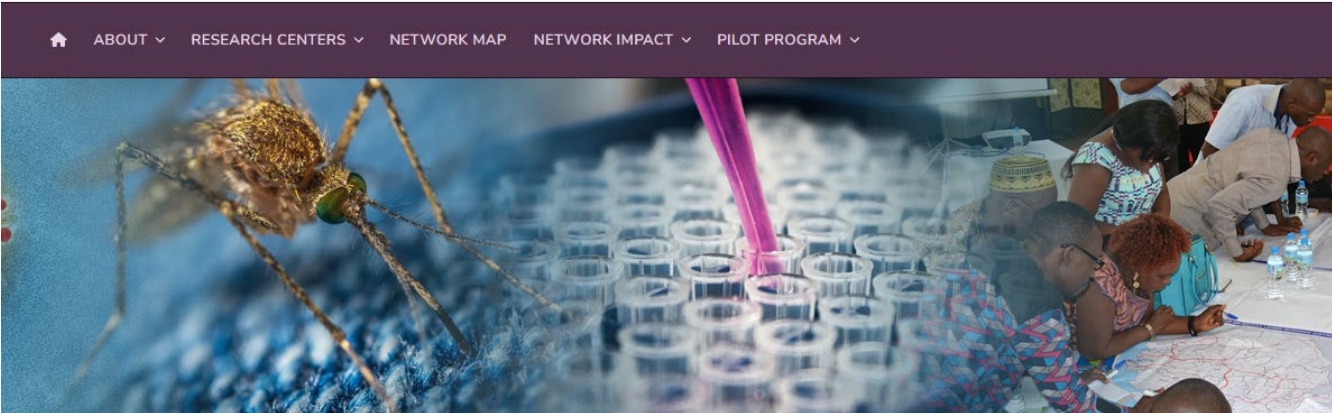
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Responding to Emerging Infectious Diseases

The Centers for Research in Emerging Infectious Diseases (CREID) Network is a coordinated group of emerging infectious disease research centers situated in regions around the globe where emerging and re-emerging infectious disease outbreaks are likely to occur. Multidisciplinary teams of investigators conduct pathogen/host surveillance, study pathogen transmission, pathogenesis and immunologic responses in the host, and develop reagents and diagnostic assays for improved detection for important emerging pathogens and their vectors. The CREID Network is developing a framework and the infrastructure necessary to respond quickly and effectively to future outbreaks.

Centers for One Health Research, Training, and Response



Fig. 1. Organization for the UTMB One Health Research, Training, and Response Hub

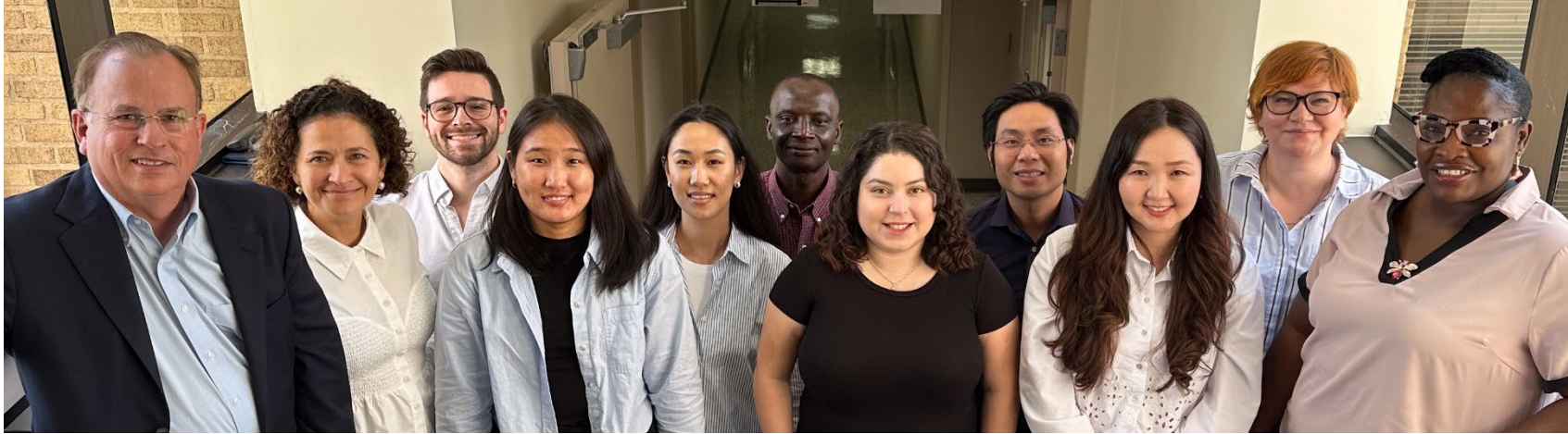
The ultimate goal of this program would be to develop independent, interdisciplinary, OH-oriented biomedical researchers.

Each Center must have the ability to offer a Master's degree in OH research and the interdisciplinary faculty (human health, veterinary health, and environmental health) to train the fellow. In addition to a Master's degree OH fellows must spend at least one year conducting a capstone research project.

Staffing - Minimum of six faculty teaching and mentoring trainees in OH with faculty in each of three disciplines: human health, animal health, and environmental health

Evidence of strong partnerships with agriculture industries

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Food Safety and Inspection Service
U.S. DEPARTMENT OF AGRICULTURE



Research: H5N1 Beef Safety Studies

Potential Research Priorities to Inform Readiness and Response to H5N1: A Workshop
National Academies of Sciences, Engineering, and Medicine
October 23, 2024

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Protect public health by preventing illness from meat, poultry, and egg products.

Our Vision

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Food Safety and H5N1 Influenza A

- USDA is confident that the meat and poultry supply are safe.
- FSIS veterinarians are available at all federal livestock slaughter facilities to ensure that animals that are unfit for human food are prevented from entering the food supply.



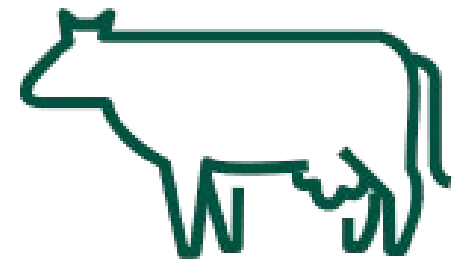
Beef Safety Studies



- Ground Beef Sampling from Retail
- Beef Muscle Sample Collection
- Beef Patty Cooking Study

H5N1 Influenza A Dairy Cow Testing Program

- On September 16, 2024, FSIS implemented an H5N1 Influenza A monitoring program for dairy cows at slaughter.
- This new program will use muscle samples already collected from dairy cow carcasses under FSIS' National Residue Program (NRP).
- As of October 17, 2024, FSIS had results from 50 muscle samples. No Influenza A has been detected in any of the samples.



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