United States Department of Agriculture

NVSL AI/ND UPDATE




JUNE 2018

DIAGNOSTIC VIROLOGY LABORATORY - AVIAN
U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
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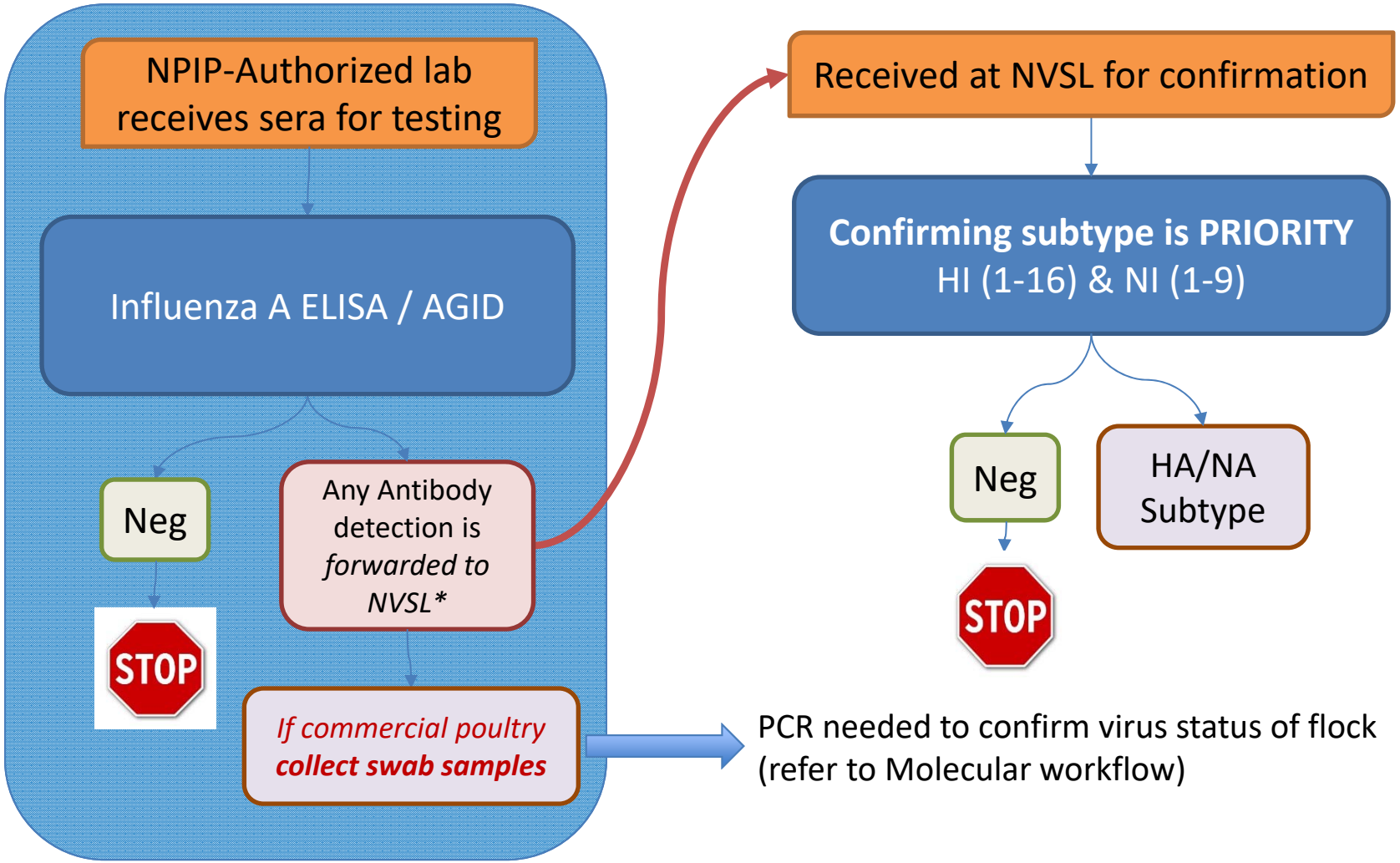
PH. 515-337-7551

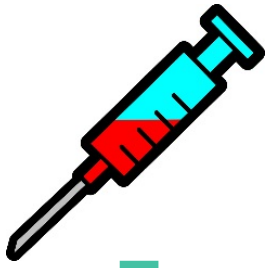
Where do my samples go…

AND WHAT DO YOU DO WITH THEM??

NPIP Serologic Workflow (AI)



Serologic Test Protocol (AI)

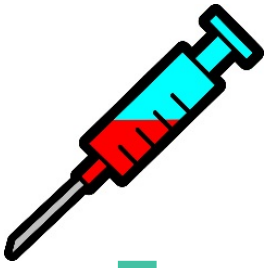


Serology informs
suspect flock status

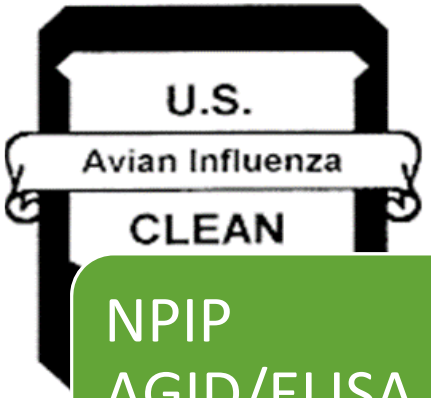
Collect swabs to determine virus status of flock

H5/H7 exposure
confirmed by NVSL HI testing

Serologic Test Protocol (AI)

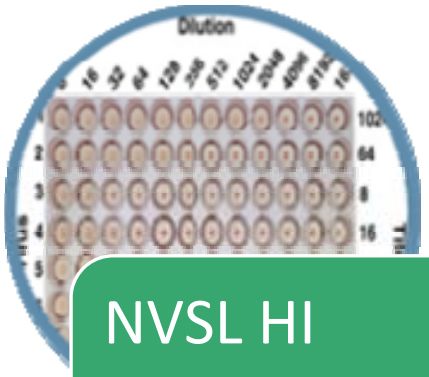


Establishes prior exposure
Test cannot determine pathotype
Test cannot determine virus status



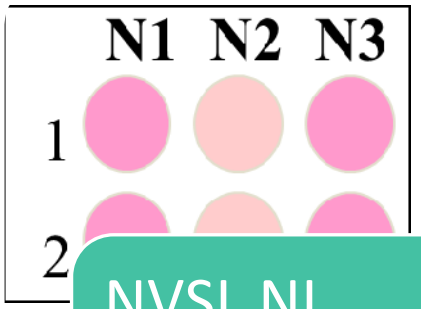
NPIP AGID/ELISA

- Detect antibody to NP



NVSL HI

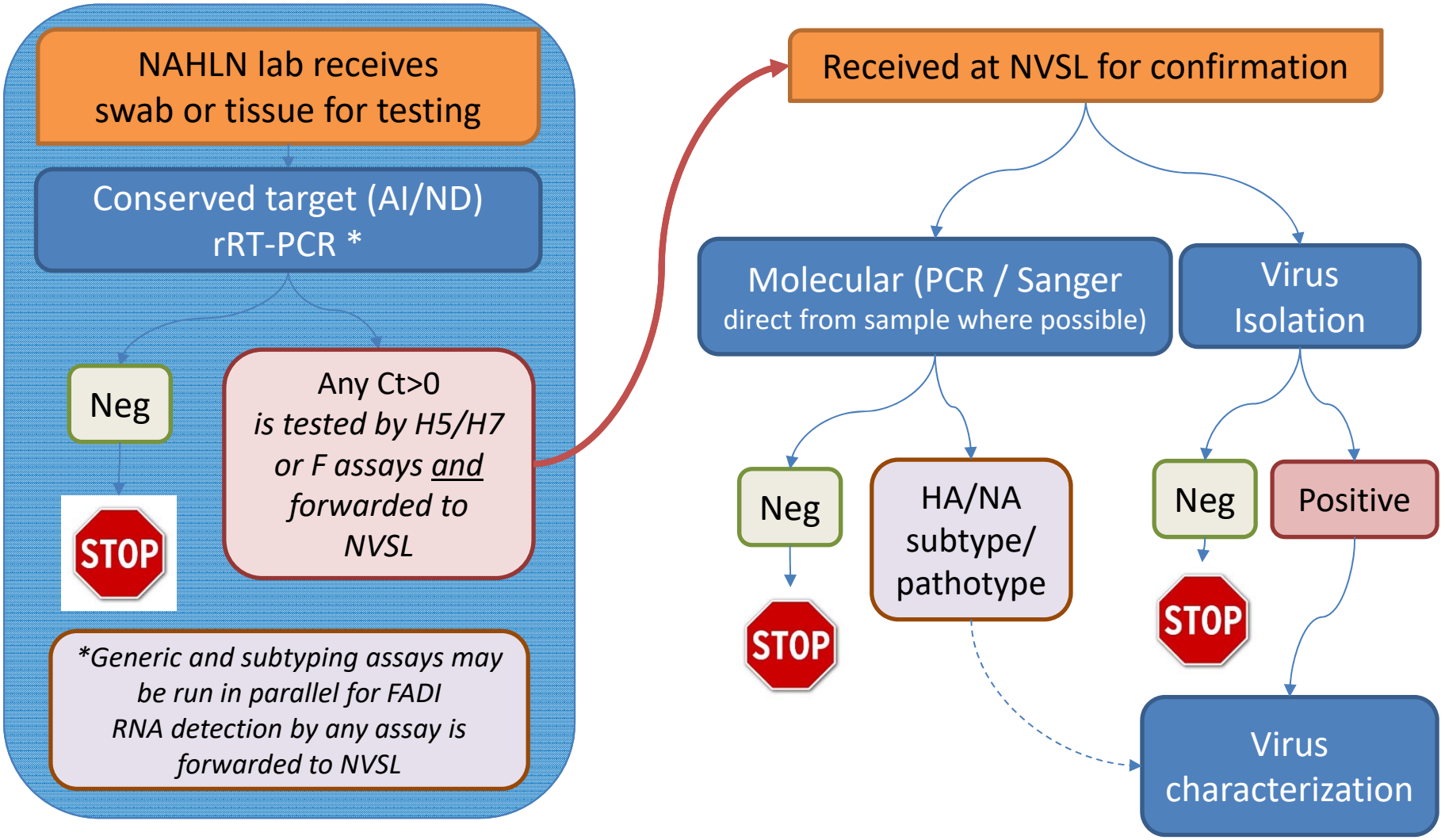
- H1-H16
- Confirm antibody to HA



NVSL NI

- N1-N9
- Confirm antibody to NA

Molecular Workflow

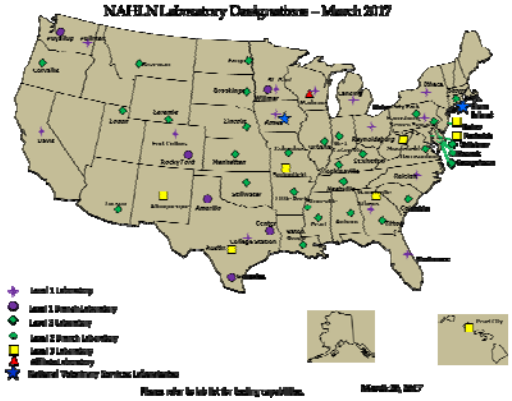


Molecular Test Protocol

PCR informs
presumptive flock status

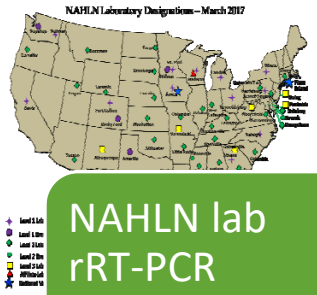
Options for rapid response available

Flock status confirmed by NVSL per USDA case definition via cleavage site sequence



Molecular Test Protocol

Disease confirmed through repeatable detection
PCR results inform risk of viral shedding
Cleavage site sequence confirms the virus



NAHLN lab rRT-PCR

- Conserved (e.g. matrix)
- Specific (H5/H7/ND)

+

NVSL repeats rRT-PCR

- Specific (H5/H7/ND)
- +/- conserved (e.g. matrix)

+

NVSL Sequence

- Partial (H&N for IAV, F for ND)
- Full genome

+

NVSL Virus Isolation

- In vivo test
- Gen/ant.char

Occurs in parallel

United States Department of Agriculture

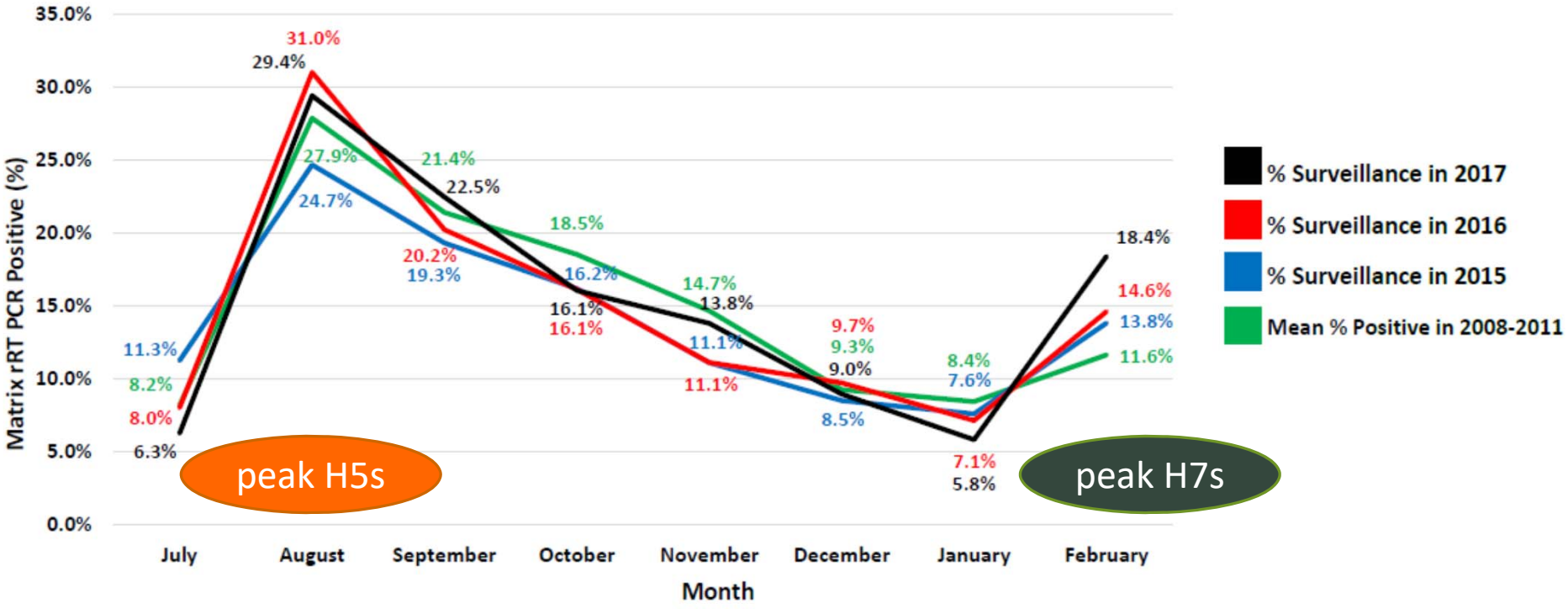
USGS science for a changing world

NATIONAL FLYWAY COUNCIL
Pacific est. 1952 - Central est. 1948 - Mississippi est. 1952 - Atlantic est. 1952

Monthly Summary Data from the National Wild Bird Avian Influenza Surveillance Program:

July 2008 to January 2018

Graph 1. Percent of wild duck samples positive for low pathogenic Type A influenza viruses as determined by RT-PCR.



Last Updated: 3/21/2018

Samples received from Interagency wild bird surveillance 2016-2018

Total wild birds sampled since Jul 2015	JUL2015–JUN2016	JUL2016–JUN2017	JUL2017–JUN2018	TOTAL
Atlantic	10,663	9,074	8,012	27,749
Mississippi	13,792	9,973	8,616	32,381
Central	9,202	7,516	7,175	23,893
Pacific	11,868	9,175	7,632	28,675
American Oceania	24	9	3	36
Total birds sampled	45,549	35,747	31,438	112,734

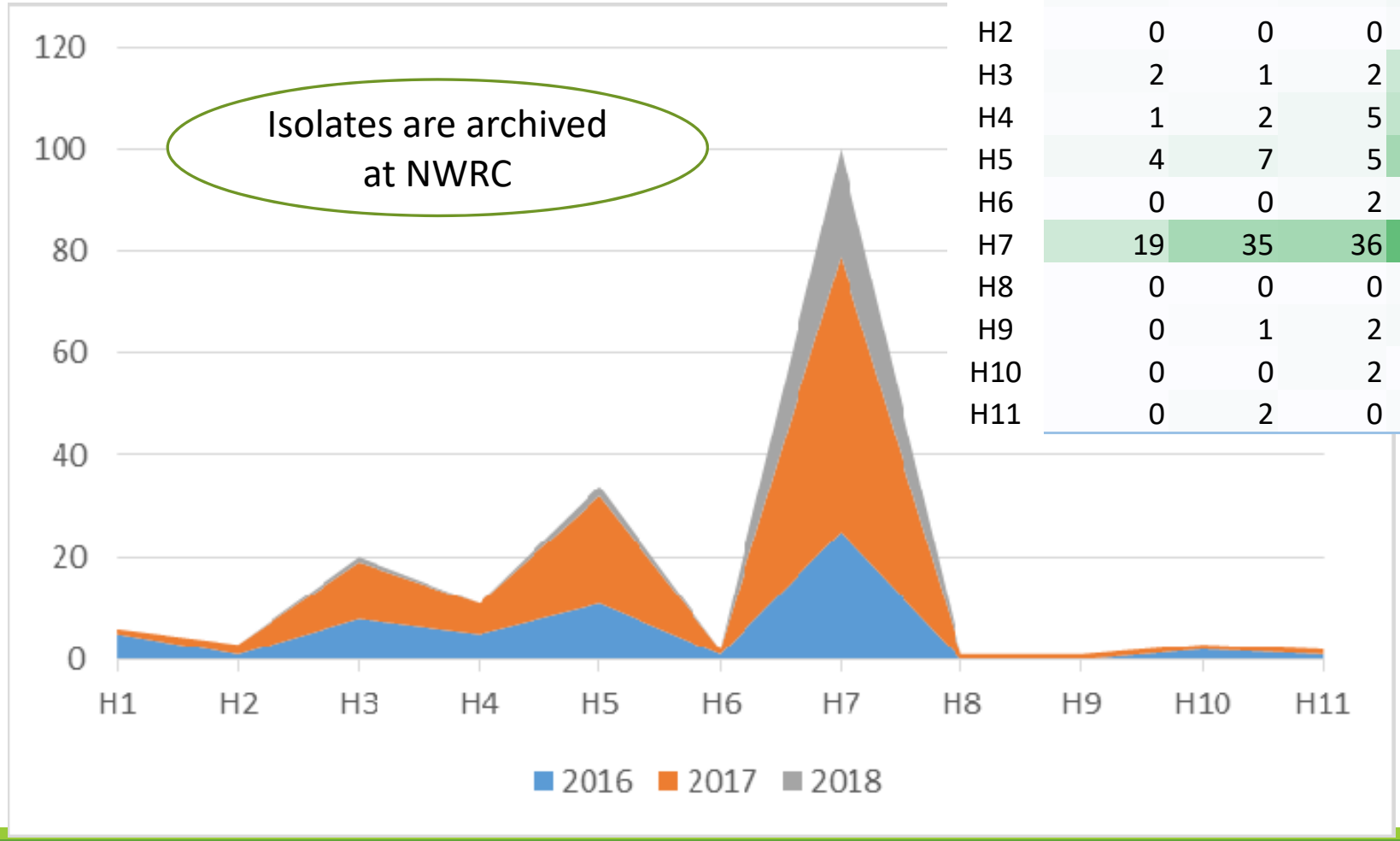
~10-11% detected by fluA PCR

Total wild birds sampled since Jul2015	JUL2015–JUN2016	JUL2016–JUN2017	JUL2017–JUN2018
Total birds sampled	45,549	35,747	31,438
avg fluA PCR +	5,010	3,932	3,458
avg H5/H7 PCR +	50	39	35

~1% detected by H5/H7 PCR go to NVSL – others to NWRC

NVSL virus recovery from Interagency Wild bird surveillance 2016-2018

flyway	ATLA	CENT	MISS	PAC
H1	2	1	2	4
H2	0	0	0	2
H3	2	1	2	20
H4	1	2	5	24
H5	4	7	5	36
H6	0	0	2	3
H7	19	35	36	62
H8	0	0	0	2
H9	0	1	2	3
H10	0	0	2	0
H11	0	2	0	2



2017-18 H5/H7 events

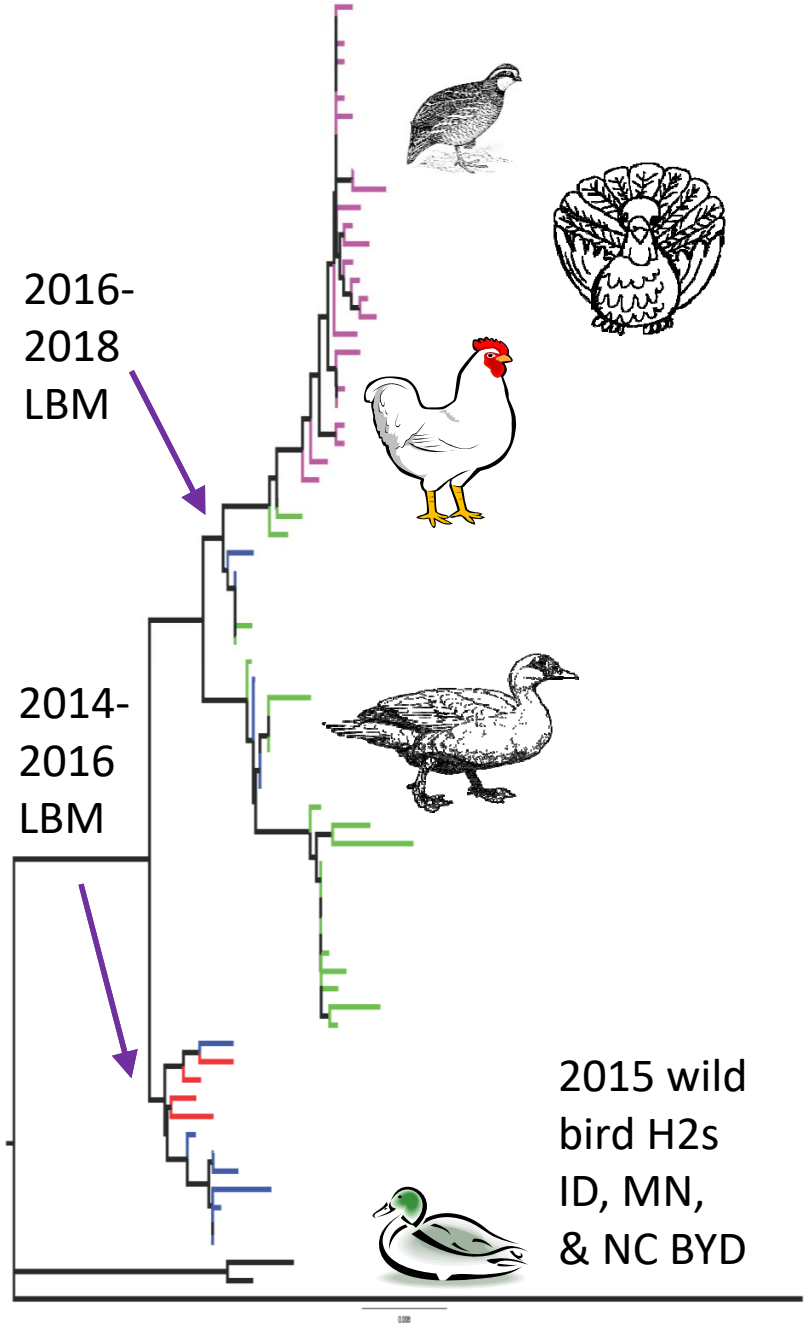
Date	State	Surv stream	initial sample	Subtype	Species	Action
Mar-17	**WI**	COMM	swab	H5N2 LPAI	turkey	Quarantine, depopulation, C&D, 10 km survellaince
Mar-17	**TN, AL, KY, GA**	COMM, BYD (AL)	swab	H7N9 HPAI/LPAI	broiler breeder (COMM), duck & guinea (BYD)	Quarantine, depopulation, C&D, 10 miles survellaince
Mar-18	**MO, TX**	COMM, BYD (MO)	swab	H7N1 LPAI	turkey, broiler breeder, chicken	Quarantine, depopulation, C&D, 10 miles survellaince
Apr-17	**ID**	BYD	sera	H5N2 LPAI	duck	Quarantine, 3km survellaince
Sep-17	**WA**	BYD	swab	H5N2 LPAI	chicken, duck	
Oct-17	**PA**	BYD	swab	H5N2 LPAI	duck	
Dec-17	**OH**	Upland Game	swab	H5N2 LPAI	duck	
Nov-17	**FL**	LBM	swab	H5 PCR only	environment	n/a

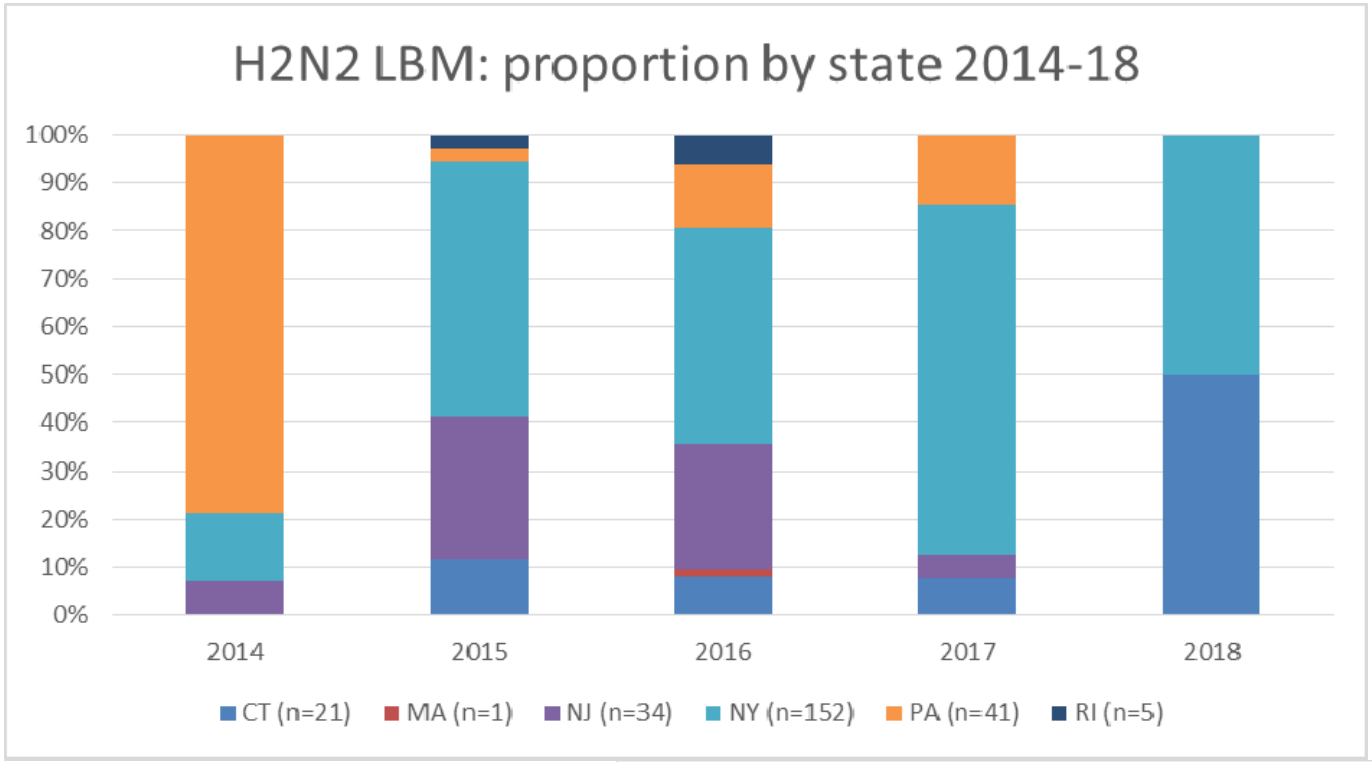
LBM H2N2 HA Phylogeny

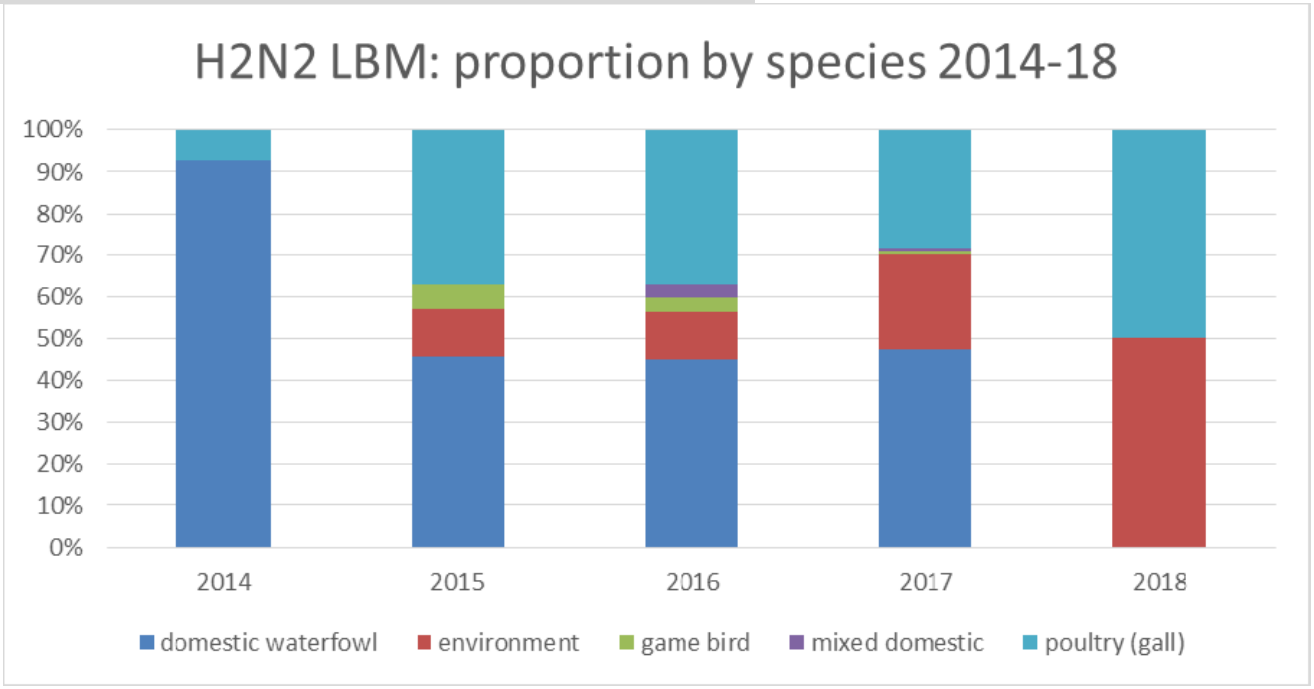
Simple tree…in general:

- Horizontal distance more significant than vertical
- Older viruses to left, younger to the right
- Red = 2014
- Blue = 2015
- Green = 2016
- Pink = 2017

Have a few H2s from wild birds -- HA not related




H2N2 LBM: proportion by state 2014-18
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2014
2015
2016
2017
2018
CT (n=21)
MA (n=1)
NJ (n=34)
NY (n=152)
PA (n=41)
RI (n=5)


H2N2 LBM: proportion by species 2014-18
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2014
2015
2016
2017
2018
domestic waterfowl
environment
game bird
mixed domestic
poultry (gall)

H2N2 - What do we know?

Wild type virus introduced to domestic waterfowl (Muscovy?) ducks prior to 2014 …

Data for viruses since 2014 suggests:

- First introduction circulated from late 2014 until 2016
- Second introduction (same virus lineage) circulating from late 2016 to present day
- Opportunity to adapt to poultry remains
- Risk of reassortment upon co-circulation with other virus remains

Not currently a public health concern – not related to previous pandemic H2s, but data are shared with CDC for ongoing evaluation

Tests *inform* Status *leading to* Response

Per the USDA case definition for AI (or ND), a suspect status is based upon the clinical presentation compared to USDA case definition with or without test result(s).

The status progresses from suspect to presumptive based upon NAHLN PCR testing, and is confirmed based upon NVSL testing.

APHIS is committed to rapid depopulation where HPAI or vNDV is suspected. Response Option: depopulation can be initiated for a presumptive premises with the following:

- The flock meets the USDA case definition,
- NAHLN H5/H7/F-assay detection (and samples that have been forwarded to NVSL for confirmation), *and*
- Agreement of State and Federal officials.

Update on vNDV in California

On May 16, 2018, the California Department of Food and Agriculture (CDFA) detected virulent Newcastle disease in a small flock of backyard exhibition chickens

USDA Confirms Additional Cases of Virulent Newcastle Disease in Backyard Birds in California

USDA Animal and Plant Health Inspection Service sent this bulletin at 06/25/2018 04:00 PM EDT

The United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service confirmed two additional cases of virulent Newcastle disease in backyard birds in San Bernardino County, California.

A complete list of confirmed cases is available on our website at www.aphis.usda.gov/animalhealth/vnd.

Virulent Newcastle disease has not been found in commercial poultry in the United States since 2003.

No human cases of Newcastle disease have ever occurred from eating poultry products. Properly cooked poultry products are safe to eat. In very rare instances, people working directly with sick birds can become infected. Symptoms are usually very mild and limited to conjunctivitis. Infection is easily prevented by using standard personal protective equipment.

General points

Newcastle Disease (ND) is the cause of regular, frequent epizootics throughout Africa, Asia, Central America, and parts of South America and is considered a Foreign Animal Disease (FAD) in the United States

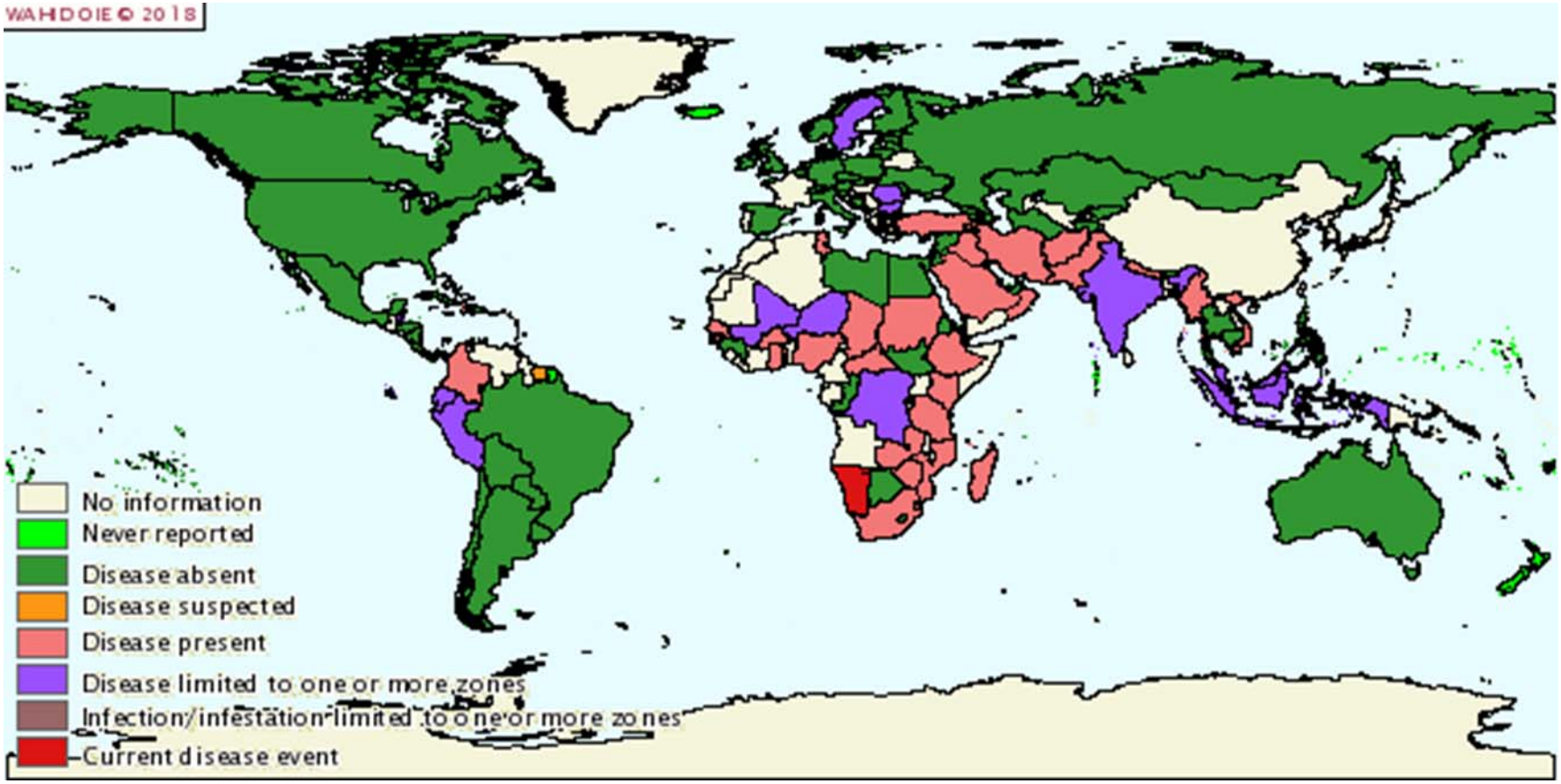
- Why not Exotic ND (END)? Because it specifically referred to viscerotropic velogenic Newcastle disease virus (vvNDV)
- Virulent ND or vND is intended to encompass all reportable virulent strains (e.g. viscerotropic and neurotropic)

The virus can infect many species of domestic and wild birds. Chickens are highly susceptible; other gallinaceous birds such as turkey, quail, and guinea are also susceptible

Vaccination of birds against ND is common in the Americas, including the United States; the classical vaccine strains are distinguishable from other viruses by genome sequencing

USDA
United States Department of Agriculture

Global Newcastle disease distribution January-July 2017 (OIE WAHIS)



Species susceptibility

Chickens are one of the most susceptible species to disease caused by vNDV; US commercial poultry are vaccinated

Turkeys are typically more resistant than chickens

Psittacine species: variable susceptibility, potential to chronically shed virus has been reported; data supporting virus maintenance in these species is lacking

Pigeons and doves in the US maintain a unique substrain (PPMV-1); unvaccinated poultry may be infected

Double-crested cormorants maintain a unique subgroup of vNDV in the US, which can infect and cause disease in poultry





Newcastle disease case definition

Suspect: Domesticated bird or flock having:

- Clinical signs compatible with vND, **OR**
- Detection of APMV-1 (e.g. matrix target) by rRT-PCR; **OR**
- Epidemiological information indicating exposure to vNDV

By NAHLN or NVSL

Presumptive: suspect + detection of vNDV by the fusion-target rRT-PCR test NOTE – a negative virulent test in the face of clinical signs requires further virus characterization by sequence and/or in vivo testing.

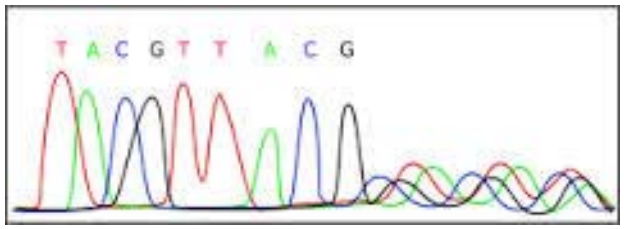
Confirmed: Domesticated bird or flock from which vNDV has been identified at NVSL

- Presumptive + identification of multiple basic amino acids (either directly via protein or by deduction through sequencing) in the fusion gene; **AND/OR**
- ICPI in day-old chicks (Gallus gallus) of 0.7 or greater

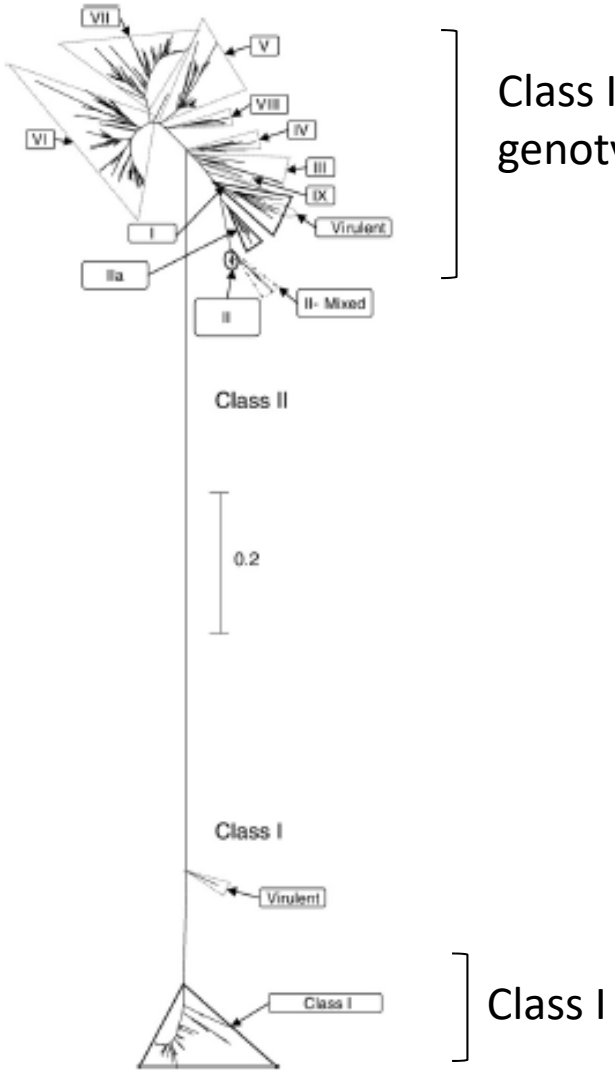
APMV-1 are: highly diverse, have a broad range of virulence, and can infect many avian species

Current diagnostics distinguish APMV-1 viruses by virulence per OIE – *Infection of poultry caused by a virus of APMV-1 that meets the following*:

- Intracerebral pathogenicity index (ICPI) of ≥ 0.7 in day-old chicks
- Presence of multiple basic amino acids at a key site in the F2 protein plus phenylalanine at residue 117 in the F1 protein
  - 'Multiple basic amino acids' = at least three arginine or lysine residues between residues 113 and 116
  - Diagnostic use of PCR to aid in detection of virulent viruses and sequencing to confirm virus lineage



Virus diversity



Class II- 19 genotypes

Class I

Class II, genotypes V, VI, VII, and VIII are the predominant worldwide and **contain only virulent viruses**

- **Class I** & **Class II, genotype I** viruses are predominantly of **low virulence** and include some used as live vaccines (QV4/66 and Ulster/67)
- **Class II, genotype II** includes viruses of low virulence and include vaccine viruses such as LaSota, B1 and VG/GA, and the neurotropic virulent chicken TX GB/1948 (challenge strain)
- Other genotypes represent historic viruses no longer seen
  - Class II, genotypes I, II, III, IV and IX represent viruses 1930– 1960

Virology 391, 2009, 64-72

Vaccination: protection from challenge

D.R. Kapczynski, D.J. King / Vaccine 23 (2005) 3424–3433

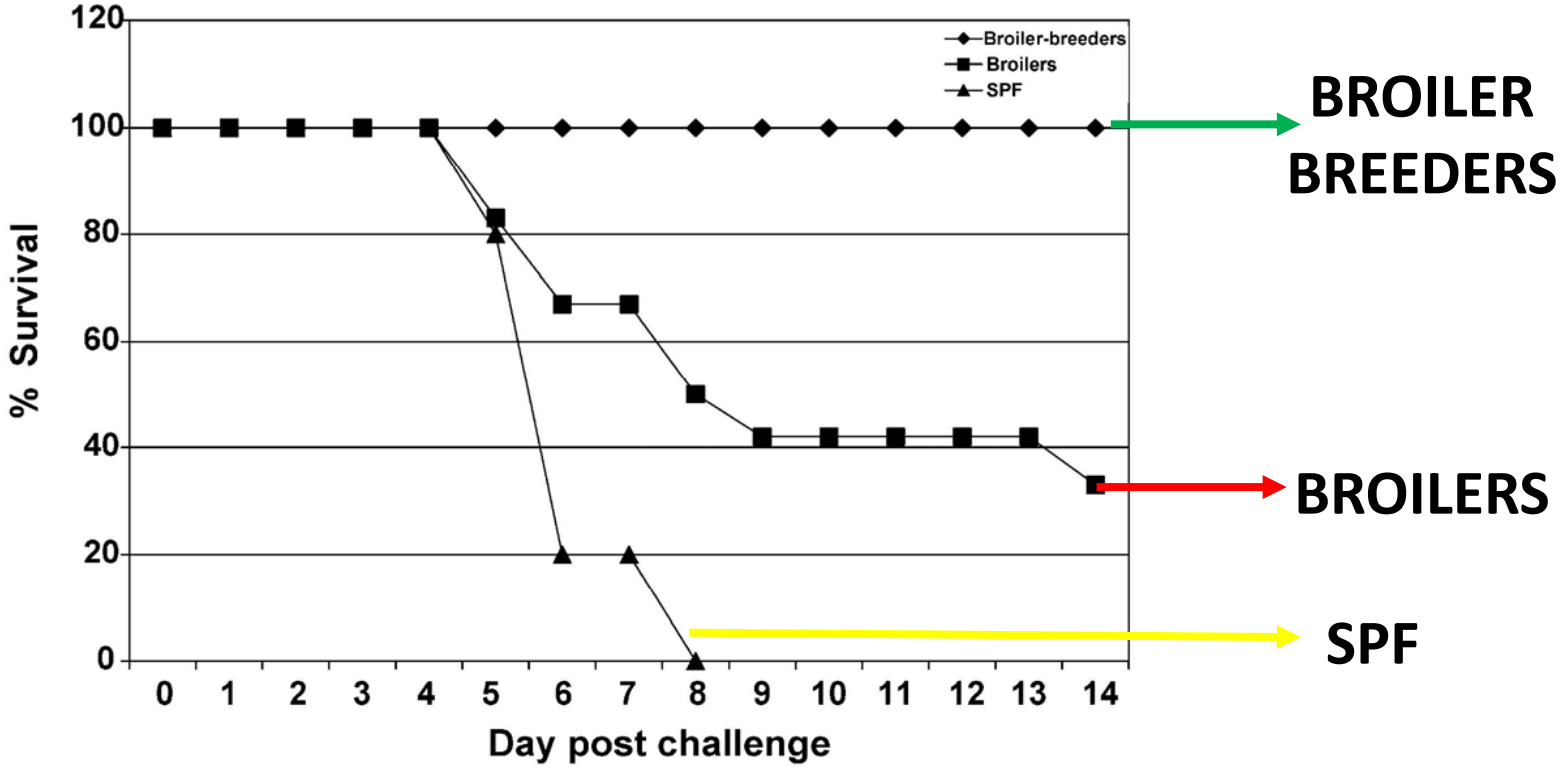
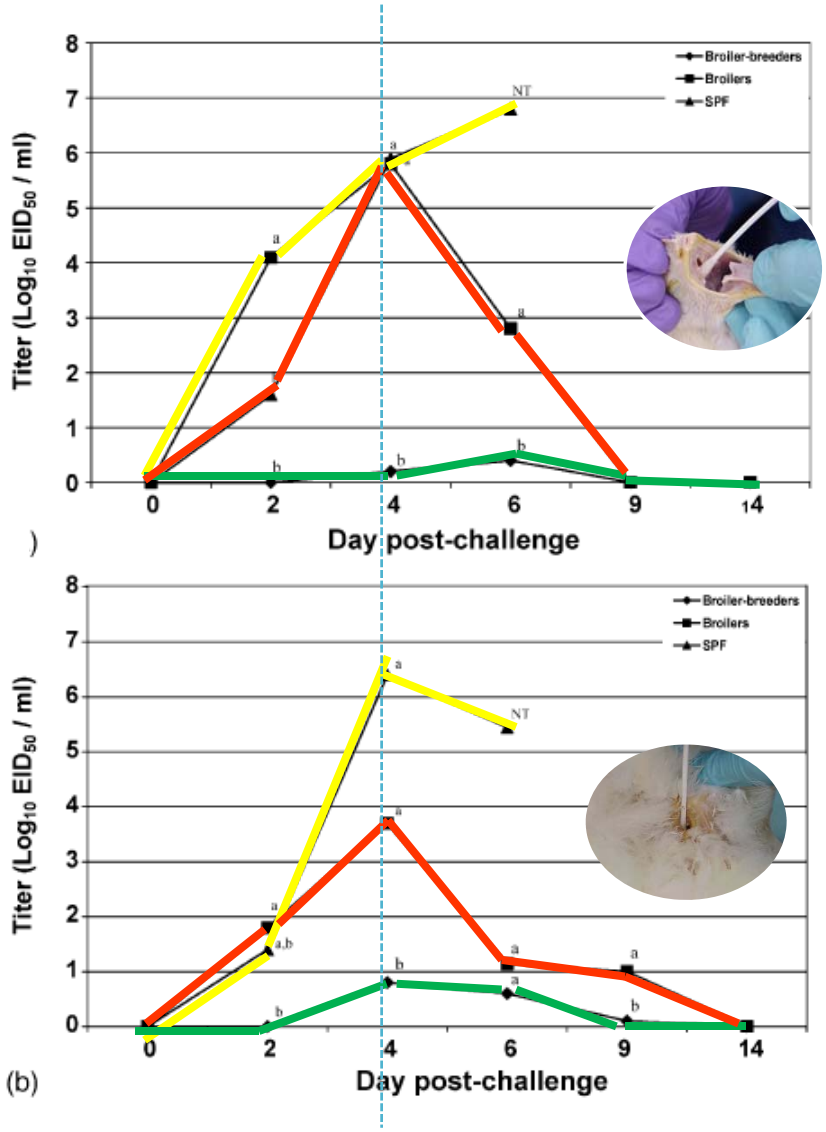


Fig. 2. Survival of commercial broiler-breeders and broilers receiving field vaccination against NDV and challenged with CA02. Chickens were infected via eye drop/intranasal route with $10^{5.9}$ EID_{50}/bird CA02 and mortality observed over a 2-week period.

Vaccination: viral shed

D.R. Kapczynski, D.J. King / Vaccine 23 (200.

Fig. 3. Comparison of CA02 mean virus titers from oral and cloacal swabs following challenge with CA02 virus in experiment III. Commercial broiler-breeders and broilers received field NDV vaccination and were challenged with $10^{5.9}$ EID_{50}/bird CA02. Oral (A) and cloacal (B) swabs were sampled on the days indicated. NT: not tested.



BROILER BREEDERS

BROILERS

SPF

COURTESY K. DIMITROV, USDA ARS SEPRL - modified

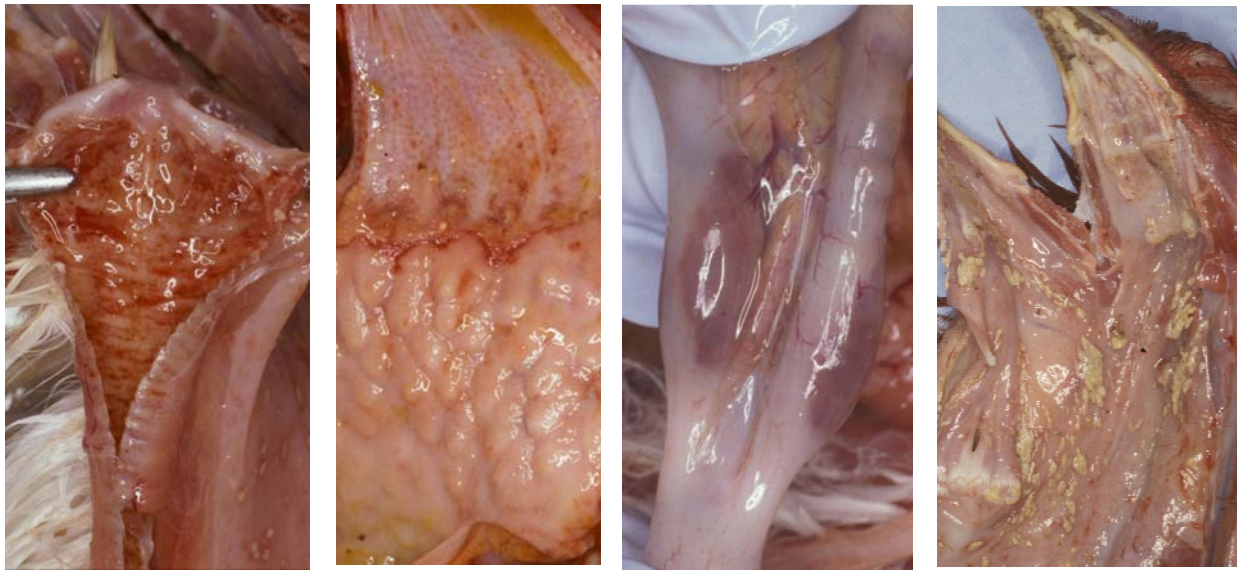
COURTESY K. DIMITROV, USDA ARS SEPRL - modified

2017 APMV-1 at NVSL

APMV-1 Diagnostic Test	Total tests 2017
Hemagglutination-inhibition (HI) antibody	2,038
Real-time RT-PCR (M, F target)	946
APMV-1 (positive/total samples)	210/3,511
Molecular pathotype (Sanger)	233
In vivo pathotype (ICPI)	49
Whole genome sequencing (count by isolate)	161

Field case definition developed for CA 2002

Defined as >2 of any 5 birds demonstrating ANY of:



- Hemorrhagic tracheitis
- Hemorrhage at junction of proventriculus
- Hemorrhage of cecal tonsils
- Diptheritic lesions

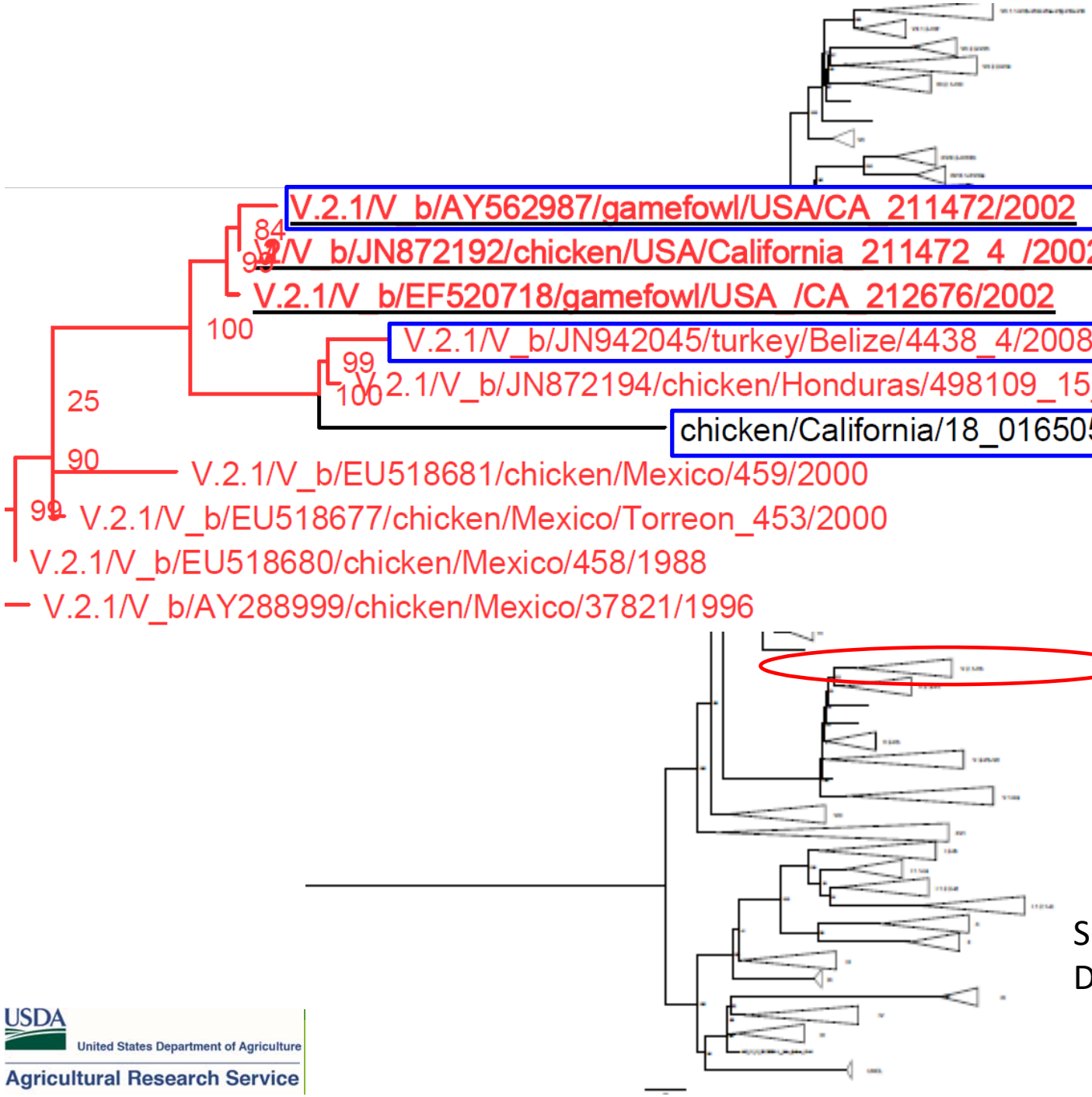
Phylogenetic analysis CA 2018

The CA 2018 virus (genotype V) is related to Mexican-lineage viruses from Central America village poultry (Belize 2008, Honduras 2007), and the U.S. (smuggled parrot 1996, backyard CA 2002).

- NOTE: temporal and geographic gaps in available data, especially from these areas and affected species, increase the uncertainty of the virus origin

The virus is not related to classic vaccine strains, vaccinated poultry strains from Mexico available (2000-2010), species-adapted viruses from columbids (pigeons, doves), nor closely related to those from cormorants.

The estimated evolutionary rate of CA 2018 compared to CA 2002 is consistent with the expected rate, and corresponds to 15-20 years of viral evolution.



SLIDE COURTESY K. DIMITROV, USDA ARS SEPRL

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Agricultural Research Service

PATHOGENESIS AND TRANSMISSION EXPERIMENT WITH THE CALIFORNIA 2018, BELIZE 2008 AND CALIFORNIA 2002 NDV

Challenge viruses:
chicken/USA/CA/212676/2002
chicken/Belize/4224-3/2008
chicken/USA/CA/016505-001/2018

Inoculation route – 0.1 mL in the choanal cleft and a touch of the gavage needle on the left eyelid

swab (OP and CL) collection from directly challenged birds daily 1 to 4 dpc, and at 7dpc

Euthanize 3 birds per day from group #10 and collect tissues – 1dpc, 2 dpc, 3 dpc

Challenge 5 birds with either 10^2, 10^4, or 10^6 EID_{50}/bird of each respective virus

Challenge 9 birds with CA18 10^6 EID_{50}/bird

3 (2) SPF contact birds added per group at 2 dpc

all contact birds swabbed (OP and CL) at 2 and 5 days post contact

CLINICAL SIGNS CONTACT 3-WEEK-OLD SPF CHICKENS

FIRST CLINICAL SIGNS APPEARED AT 4 DAYS POST CONTACT

	Eyelid edema	Conjunctivitis	Lethargy
CA/02 10^2	-	-	Yes
CA/02 10^4	Yes	Yes	Yes
CA/02 10^6	Yes	Yes	Yes
Belize/08 10^2	-	-	-
Belize/08 10^4	Yes	Yes	Yes
Belize/08 10^6	Yes	Yes	Yes
CA/18 10^2	-	-	-
CA/18 10^4	Yes	Yes	Yes
CA/18 10^6	Yes	Yes	Yes

-: not observed

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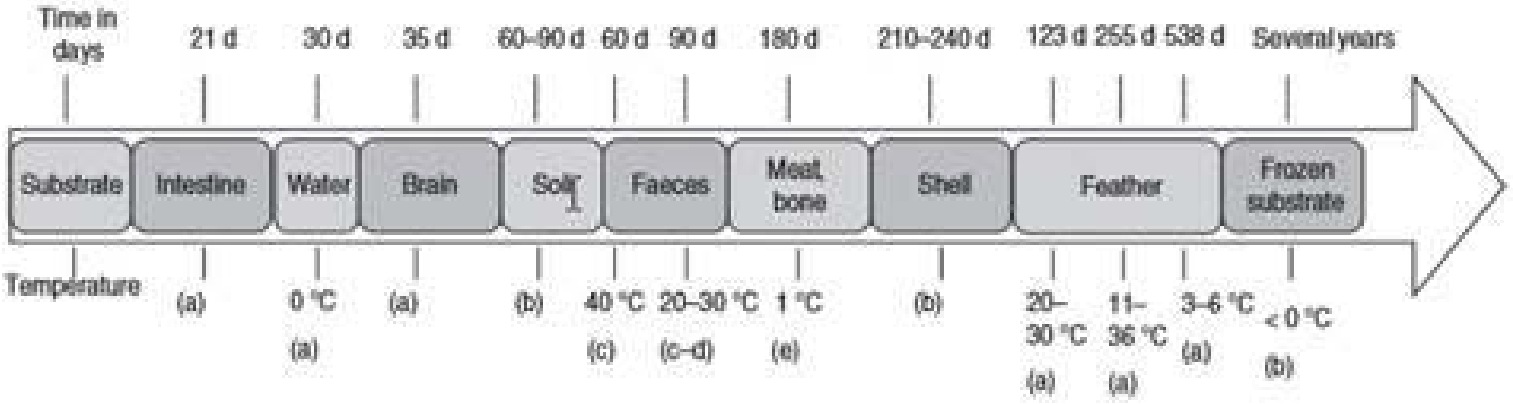
SLIDE COURTESY K. DIMITROV, USDA ARS SEPRL

NDV survival in the environment



Incubation period (domestic chicken) (f)	Ordinary 4–6 d	Minimum 2–6 d	Maximum 15–21 d
Mode of transmission (g)	Faecal–oral ++	Respiratory +	
pH (f)	3–11 Survival 1 week		
Inactivation temperature (a–f)	(°C) 60 °C 56 °C	Time (h) 0.5 h 3 h	

Epidemiol. Infect. (2013), 141, 1117–1133. Cambridge University Press 2012 doi:10.1017/S0950268812002610

Fig. 1. Newcastle disease and virus eco-epidemiological characteristics: survival of the virus for different substrates, temperatures and pHs; incubation periods and transmission modes. (a) [17], (b) [95], (c) [8], (d) [96], (e) [97], (f) [1], (g) [4].

NDV survival in chicken carcasses

Chickens inoculated with virulent NDV (Pakistan/VIIi) and euthanized when clinical signs were apparent (~3-4 days post challenge)

Carcasses were stored at ambient temperatures (~75°F) for 18 days

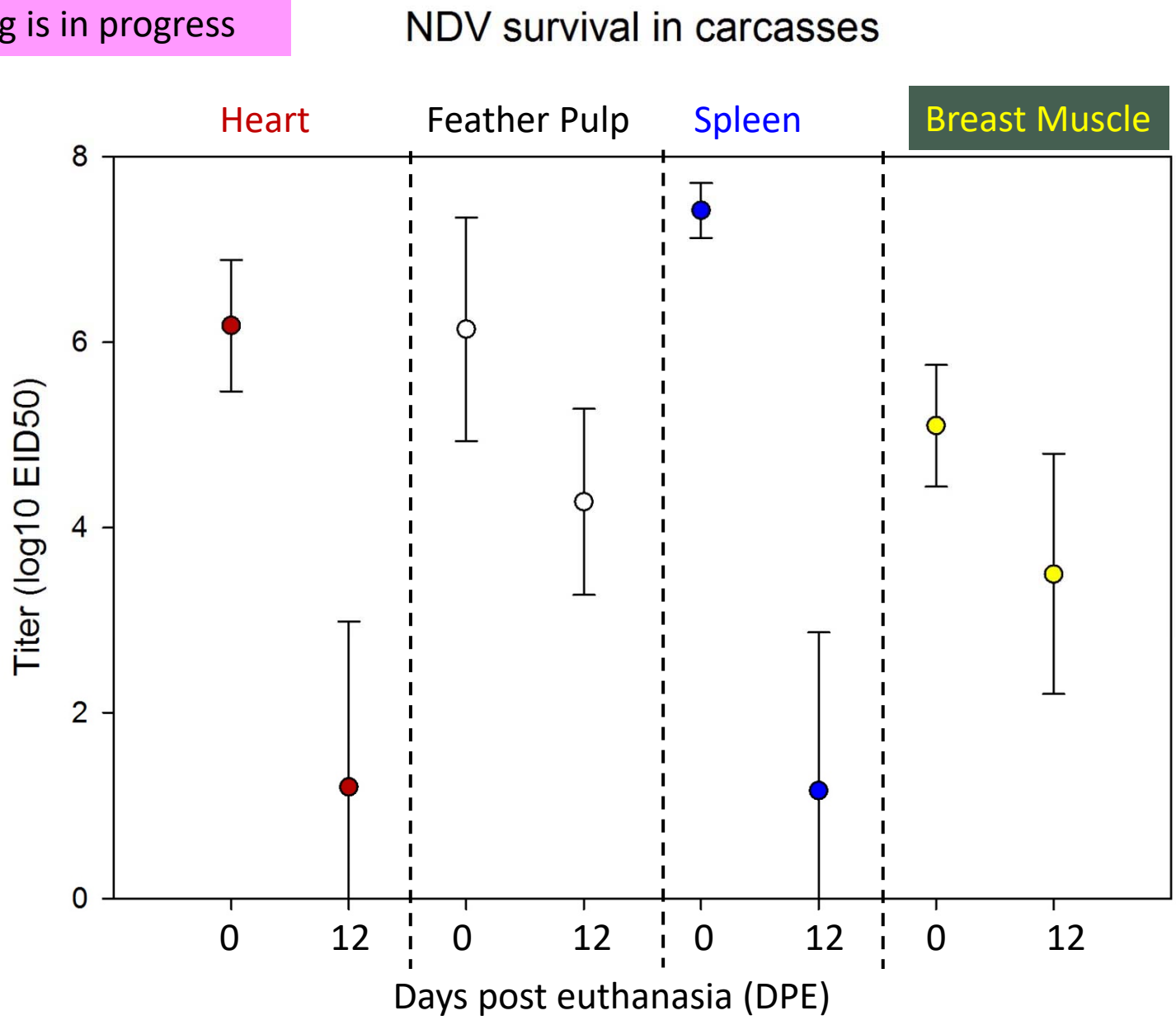
8 tissues and fluid draining from the carcasses were collected at the time of euthanasia and at 6, 12, and 18 days post euthanasia (DPE)

- Heart, spleen, feather pulp, breast muscle, thigh muscle, skin, small intestine, lung, fluid
  - Each sample is processed for virus viability and titer
    - Only some tissues have been tested at 12 DPE
    - In all tested tissues viable virus has been detected

Preliminary data, sample processing is in progress

DATA COURTESY E. SPACKMAN, USDA ARS SEPRL

Preliminary data, sample processing is in progress



USDA United States Department of Agriculture
Agricultural Research Service

DATA COURTESY E. SPACKMAN, USDA ARS SEPRL

Helpful References

Other helpful references

- **Newcastle Disease Brief**
  [English PDF]
- **Newcastle Disease Fact Sheet**
  [English PDF]
- **Comparison Chart for HPAI and Newcastle Disease**
  [English PDF]
- **Biosecurity and Disinfection Disinfection 101**
  [English PDF]
- **Characteristics of Selected Disinfectants**
  [English PDF]

Special Thanks to:

- CAHFS
- USDA ARS SEPRL
- SAHOs, NPIP and NAHLN Labs
- Team Avian, Diagnostic Virology Lab

Remember to send duplicate samples to your NAHLN lab and to NVSL in parallel for the fastest confirmation!

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