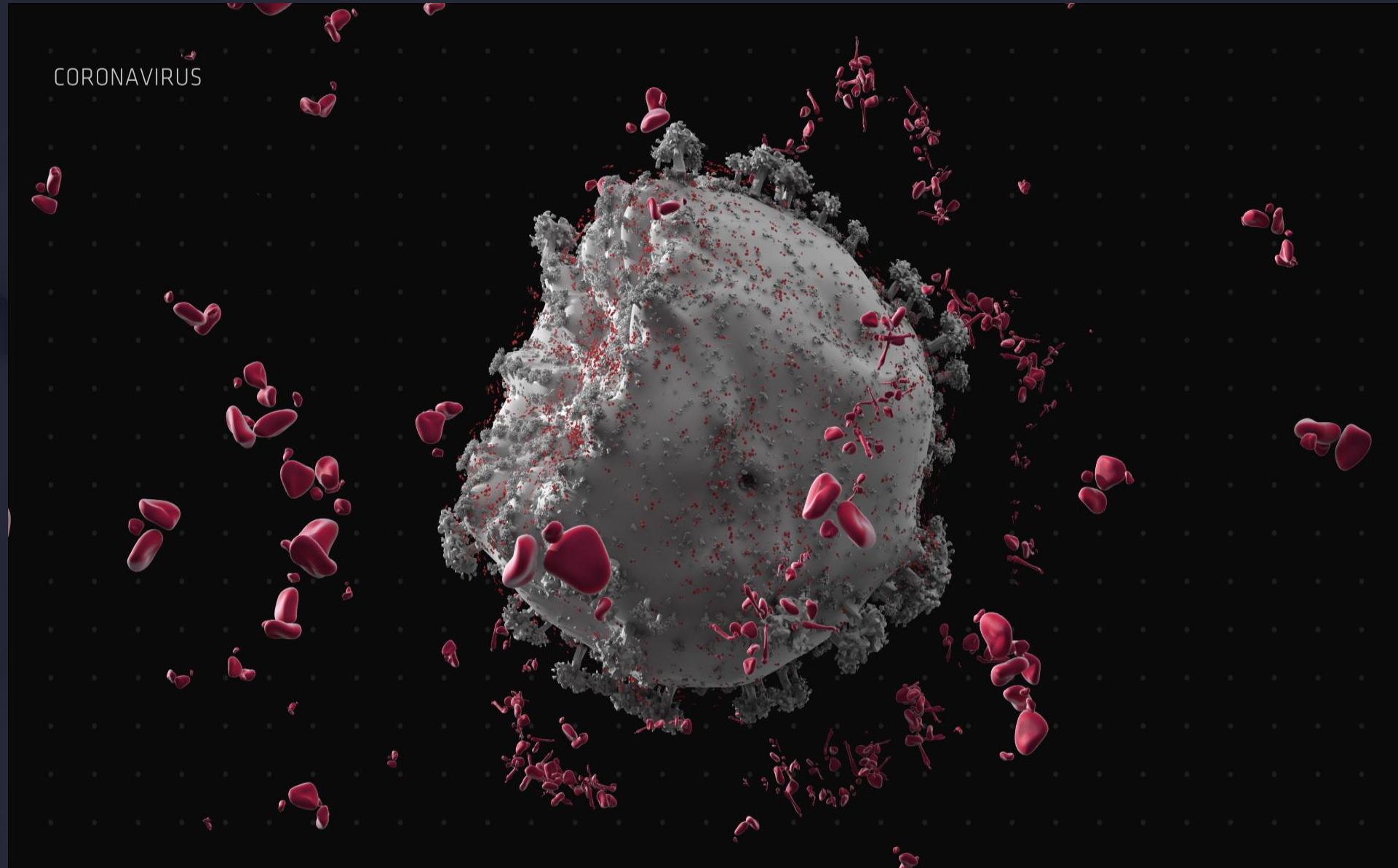


Structured Molecular Diagnostic
Sampling Strategies Optimize
Vaccination Based Control of
Infectious Bronchitis in Poultry

Dr. Aurora Romero Tejeda
x-OvO Limited

The Genetics of Infectious Bronchitis Change as a Normal Part of Virus Biology

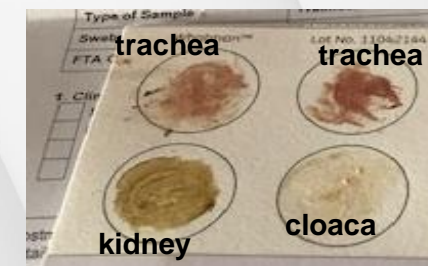


Samples to Collect



	Vaccine Take	Vaccine Persistence	Clinical IB investigation or clear clinical signs
TRACHEA	+++ ⁽¹⁾		+ ⁽²⁾
CLOACA	+++	+++	+++
KIDNEY	+ ⁽³⁾	+ ⁽³⁾	++ ⁽⁴⁾

- 1 - In particular for Mass-type vaccines (7-14 days post vaccination)
- 2 - Only if ongoing or recent respiratory signs observed
- 3 - Some vaccines (4/91 strain) may be good colonisers of the kidney
- 4 - Only if nephritis or kidney problems observed/detected in the farm

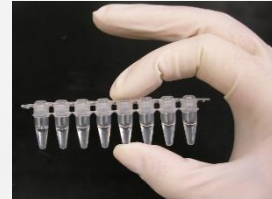


AVOID collecting organs where IB virus is not present in major quantities (e.g. bursa, spleen, liver, pancreas, oviduct, uterus, brain, skin, eye, etc.).



AVOID mixed sampling site pool – higher possibility of false negative or untypeable findings (dilution effect)

The Diagnostic Pathway Reads and Quantifies Infectious Bronchitis Genetic Information



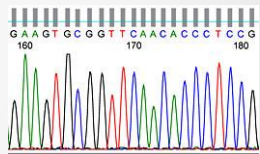
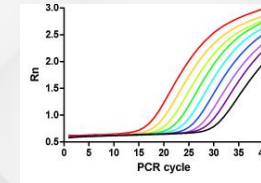
Real time RT-PCR



Is IB present YES or NO



How much is present?



Sequencing PCR (S1 gene) and amino acid alignments



Differentiation of vaccines from field viruses



Interpretation, reporting and recommendations

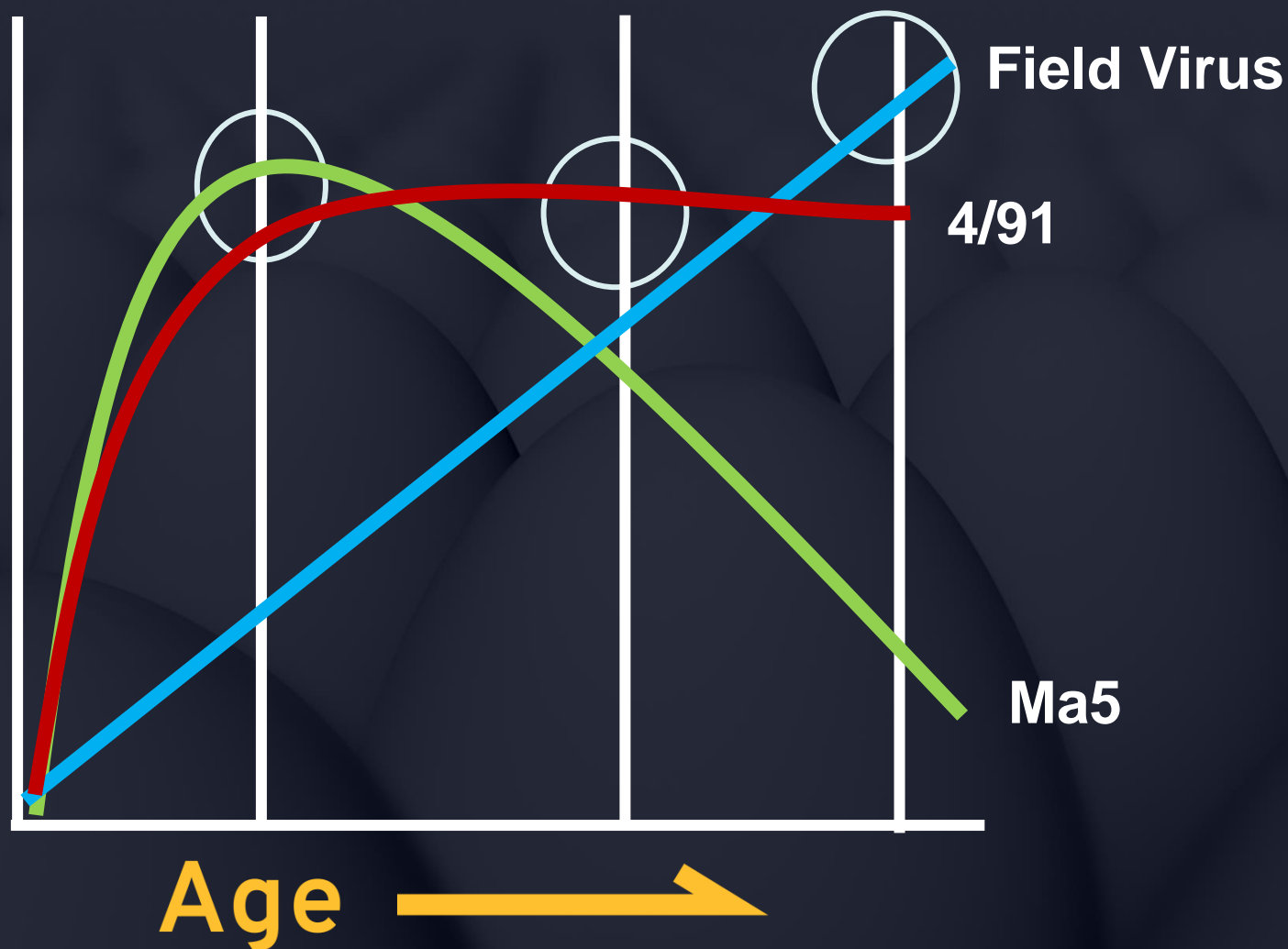
Effective IB Control Requires Accurate Data from Structured Diagnostic Sampling

- 1 We need to know the normal replication kinetic of vaccines**
Measurable with DOMINANT STRAIN SEQUENCING AND HIGHLY SPECIFIC REAL TIME PCR
- 2 We need to know if vaccines were given to the flock as intended**
Measurable with HIGHLY SPECIFIC REAL TIME PCR
- 3 We need to know the genetic 'finger-print' of any dominant field virus present**
Sanger Sequencing – Dominant Strain Characterization

Big data set references to differentiate vaccine origin viruses from sequences of probable field virus origin
- 4 We need to ensure that timing of onset of immunity following vaccination occurs in advance of the arrival of the field challenge**

1

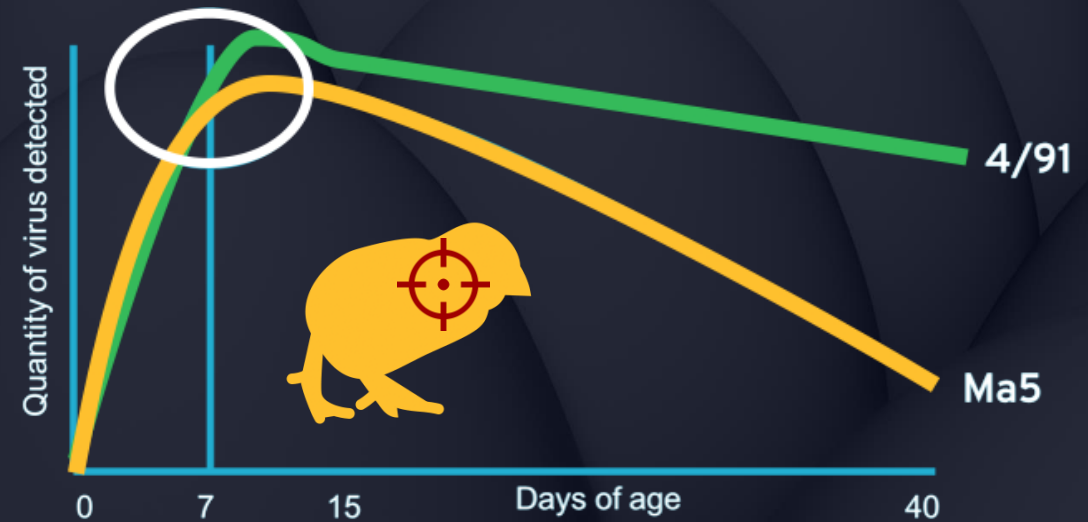
Live Vaccines and Field Viruses Replicate and **Decay Dynamically**



2

The ProtectoTake Test (PTT) Quantifies the Presence of Ma5 plus 4/91 Genotype Vaccines in a Single Sample

Both vaccine components regularly recovered from the trachea at young ages. The quantity of Mass genotype vaccine is greater in tracheal samples hence pooled tracheal samples from 10 birds, collected at day 7 post vaccination are the recommended sample to evaluate vaccine take.



The Sample of Choice is the Trachea Seven Days After Vaccination

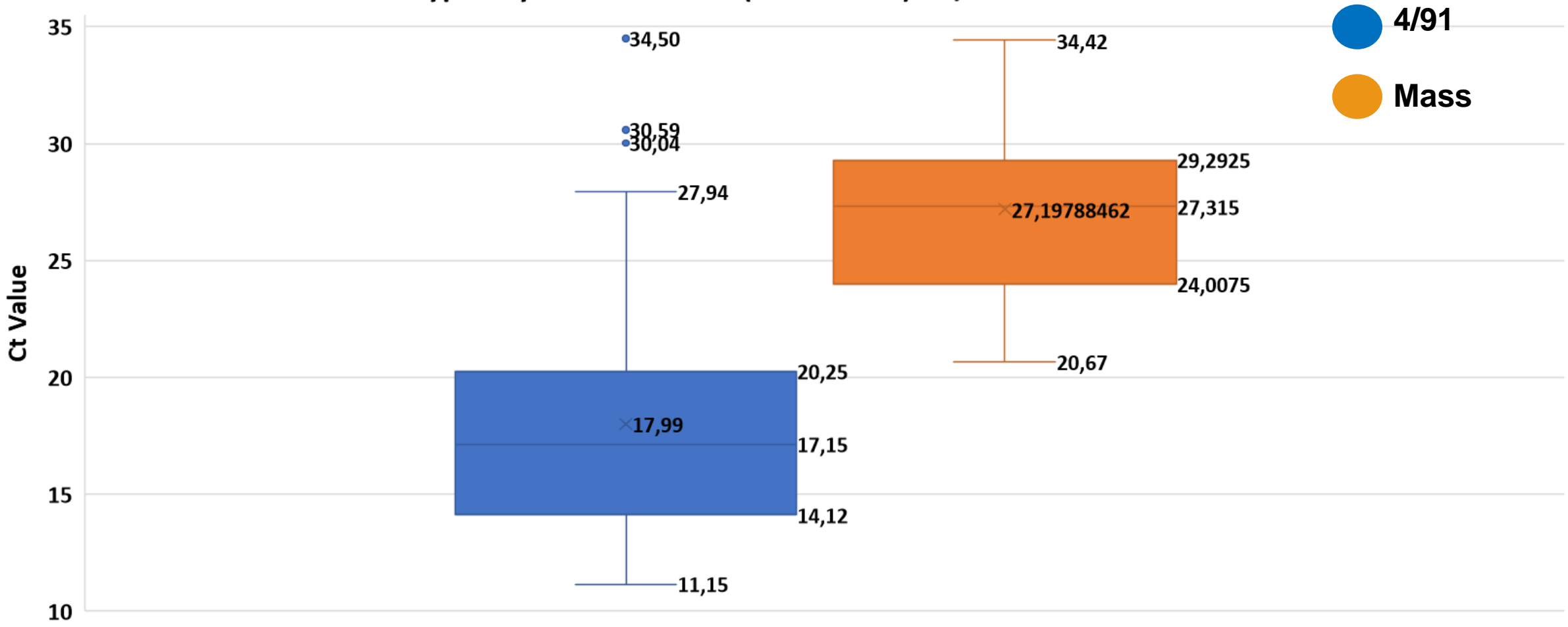
Key Fact

Live infectious bronchitis vaccines must replicate in the chicken to create an immune response

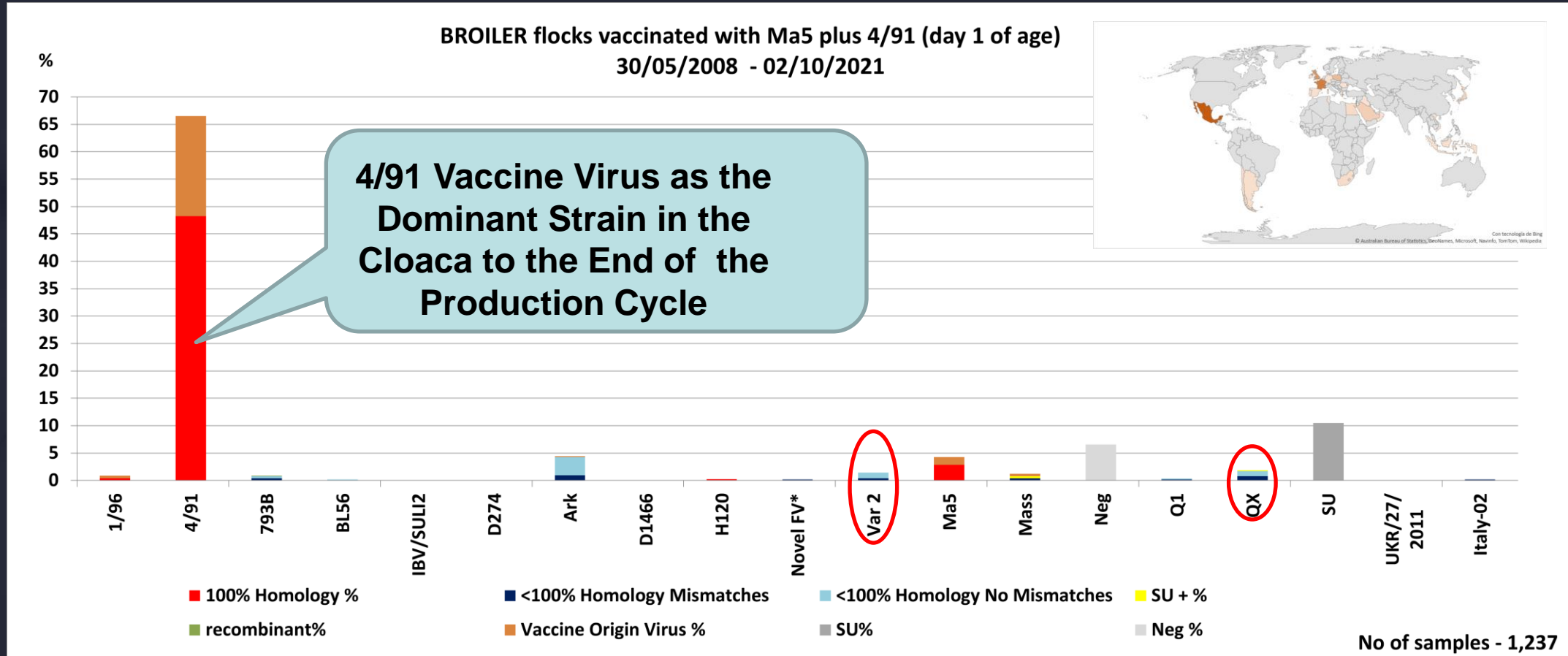
Broilers vaccinated with 4/91 and Ma5 vaccine genotypes at day 1



Protectotype day 1 - PTT Results (n=57 flocks) - 4/91 and Mass Ct values



QX And Var 2 Field Virus Detection Is Very Rare With Day of Age Ma5 plus 4/91 Genotype Vaccination



Number of flocks : 1,038. Countries : 28

Any sample collected was included: trachea, cloaca, mixed, etc.

Age between 1 and 99 days (average 35.2 days)

Countries: Argentina, Baltics, Belgium, Chile, Cyprus, Greece, Egypt, France, Germany, Indonesia, Iraq, Ireland, Japan, Mexico, Middle East, Netherlands, Oman, Poland, Portugal, Romania, Saudi Arabia, S. Africa, Spain, Taiwan, Tunisia, Turkey, UK, Vietnam.

*Novel FVs more closely related to some Brazilian IB strains (BIBREMBRAPA/116, USP 589-5)

3

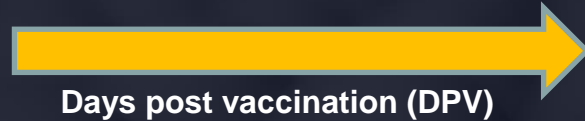
Next Generation Sequencing and Big Data Facilitate Virus Strain Differentiation

The different sub-populations in a vaccine vial can undergo selection in chickens after vaccination (some of them may become dominant and some disappear).

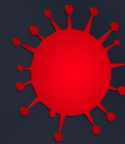


Minor sub population (s) ● ●
Major population ●

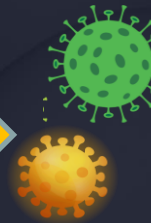
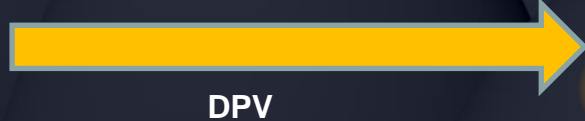
Vaccine



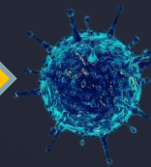
Sampling



Recovery at a known time point PV of a strain 100% homologous to the vaccine take population.



Viruses (<100% homology to the vaccine take population) homologous to defined genetic sub-populations in the vaccine vial



The vaccine sub-populations can further mutate (to improve their replication). These mutations are different than those present in the vaccine sub-populations. XOvO has a big data set containing all these mutations that are NORMALLY observed in vaccinated flocks without displaying clinical signs.

Protectotype Vaccines Can be Detected as **Dominant Strains** X-●VO in Cloacal and Tracheal Samples in Broilers

Origin of Samples			Age at Sampling	Type of Birds
Tracheal	Cloacal	Other	35 day	broiler
10	10			

Clinical Symptoms /Reason for Submission			
<input type="checkbox"/>	Decreased feed/water intake	<input type="checkbox"/>	Mild/Severe respiratory problems
<input type="checkbox"/>	Birds depressed	<input type="checkbox"/>	Sneezing, spitting, rales
<input type="checkbox"/>	Poor growth	<input type="checkbox"/>	Tracheitis
<input checked="" type="checkbox"/>	Routine monitoring	<input type="checkbox"/>	Conjunctivitis
		<input type="checkbox"/>	Swollen heads / Nasal exudate / Sinusitis
		<input type="checkbox"/>	Increased mortality
		<input type="checkbox"/>	Wet litter / Enteritis / Scour
		<input type="checkbox"/>	Decrease in shell quality
		<input type="checkbox"/>	Drop in egg production
		<input type="checkbox"/>	Nephritis / Kidney problem

Vaccination History			
Vaccine	Age	Dose	Route of Application
4-91	1 d	1 ds	Coarse spray
Ma5	1 d	1 ds	Coarse spray

Molecular Analysis	Sample 1 (tracheal) Molecular analysis identified a large quantity of IB viral RNA (Ct =22.81).		
Sequencing Analysis	Sample 1 (tracheal) - Sequencing analysis characterised this virus as a Mass type infectious bronchitis virus with 100% nucleic acid sequence homology and 100% amino acid sequence homology to the known Ma5 vaccine vial sequence:		
Nucleic Acid Sequence – Sample 1	100% Homologous to Ma5 Vaccine Vial		
Position	Vaccine Vial Sequence	Sample Sequence	Comments

Molecular Analysis	Sample 2 (cloacal) Molecular analysis identified a significant quantity of IB viral RNA (Ct = 25.35)		
Sequencing Analysis	Sample 2 (cloacal) - Sequencing analysis characterised this virus as a 4/91 infectious bronchitis virus with 100% nucleic acid sequence homology and 100% amino acid sequence homology to the known 4/91 vaccine vial sequence:		
Nucleic Acid Sequence – Sample 2	100% Homologous to 4/91 Vaccine Vial		
Position	Vaccine Vial Sequence	Sample Sequence	Comments



Ma5 VV in the **TRACHEA** and a **4/91** VV in the **CLOACA** demonstrating the vaccine take of the vaccines applied at day one of age

Dual Sampling of Trachea and Cloaca Can Demonstrate Field Challenge (WITH Clinical Signs)

Origin of Samples			Age at Sampling	Type of Birds
Tracheal	Cloacal	Other	28 day	broiler
10	10			

Clinical Symptoms /Reason for Submission			
<input type="checkbox"/>	Decreased feed/water intake	<input type="checkbox"/>	Mild/Severe respiratory problems
<input type="checkbox"/>	Birds depressed	<input type="checkbox"/>	Sneezing, snicking, rales
<input checked="" type="checkbox"/>	Poor growth	<input type="checkbox"/>	Tracheitis
<input type="checkbox"/>	Routine monitoring	<input type="checkbox"/>	Conjunctivitis
		<input type="checkbox"/>	Swollen heads / Nasal exudate / Sinusitis
		<input checked="" type="checkbox"/>	Nephritis / Kidney problems
<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	Increased mortality
		<input checked="" type="checkbox"/>	Wet litter / Enteritis / Scourir
		<input type="checkbox"/>	Decrease in shell quality
		<input type="checkbox"/>	Drop in egg production

Vaccination History			
Vaccine	Age	Dose	Route of Application
4-91	1d, 14d		Coarse spray
Ma5	1d		Coarse spray

Molecular Analysis Sample 1 (tracheal) - Molecular analysis identified a very large quantity of IB viral RNA (Ct = 16.15).

Sequencing Analysis Sample 1 - Sequencing analysis characterised this virus as a Var 2 infectious bronchitis virus with the following substitutions from the known Var 2 vaccine vial sequence.

Nucleic Acid Sequence – Sample 1			98% Homologous to Var 2 Vaccine Vial
Position	Vaccine Vial Sequence	Sample Sequence	Comments
243	Cytosine	Thymine	

Molecular Analysis Sample 2 (cloacal) - Molecular analysis identified a large quantity of IB viral RNA (Ct = 24.05).

Sequencing Analysis Sample 2 - Sequencing analysis characterised this virus as a 4/91 infectious bronchitis virus with 100% nucleic acid sequence homology and 100% amino acid sequence homology to the known 4/91 vaccine vial sequence:

Nucleic Acid Sequence – Sample 2			100% Homologous to 4/91 Vaccine Vial
Position	Vaccine Vial Sequence	Sample Sequence	Comments



Var 2



ACTION:
Check Vaccine Application

Var 2 FV in trachea and 4/91 VV in the cloaca

The result suggests:

FV arrived in advance of the onset of immunity

OR

A possible problem with the application of Ma5 component of the vaccination programme

4 Onset of Immunity is Delayed by Divided Doses or Late Vaccine Administration



Field Example of Early IB Challenge and Sub Optimal Vaccination



Origin of Samples			Age at Sampling	Type of Birds
Tracheal	Cloacal	Other	6 day	broiler
20				

Clinical Symptoms /Reason for Submission			
<input type="checkbox"/>	Decreased feed/water intake	<input type="checkbox"/>	Mild/Severe respiratory problems
<input type="checkbox"/>	Birds depressed	<input type="checkbox"/>	Sneezing, snicking, rales
<input type="checkbox"/>	Poor growth	<input type="checkbox"/>	Tracheitis
<input type="checkbox"/>	Routine monitoring	<input type="checkbox"/>	Conjunctivitis
		<input type="checkbox"/>	Swollen heads / Nasal exudate / Sinusitis
		<input type="checkbox"/>	Increased mortality
		<input type="checkbox"/>	Wet litter / Enteritis / Scouring
		<input type="checkbox"/>	Decrease in shell quality
		<input type="checkbox"/>	Drop in egg production
		<input type="checkbox"/>	Nephritis / Kidney problems

Vaccination History			
Vaccine	Age	Dose	Route of Application
	4-91	Day one	1
	Ma5 +	Day one	1
		Embryo D18	1
		Embryo D 18	1
			Spray
			Spray
			IN OVO
			IN OVO

Molecular Analysis	RESULTS
Mass Real time RT-PCR	Negative
793B Real time RT-PCR	4/91 Positive (Ct= 21.90)

Molecular Analysis	Molecular analysis identified a large quantity of IB viral RNA (Ct = 23.08). NDV (real time PCR) – Positive (Ct=27.70)
---------------------------	--

Sequencing Analysis	Sequencing analysis characterised this virus as a IBV/SULI2/2017 infectious bronchitis virus with the following substitutions from the known IBV/SULI2/2017 (GenBank MF806473, Var 2-like) reference strain:
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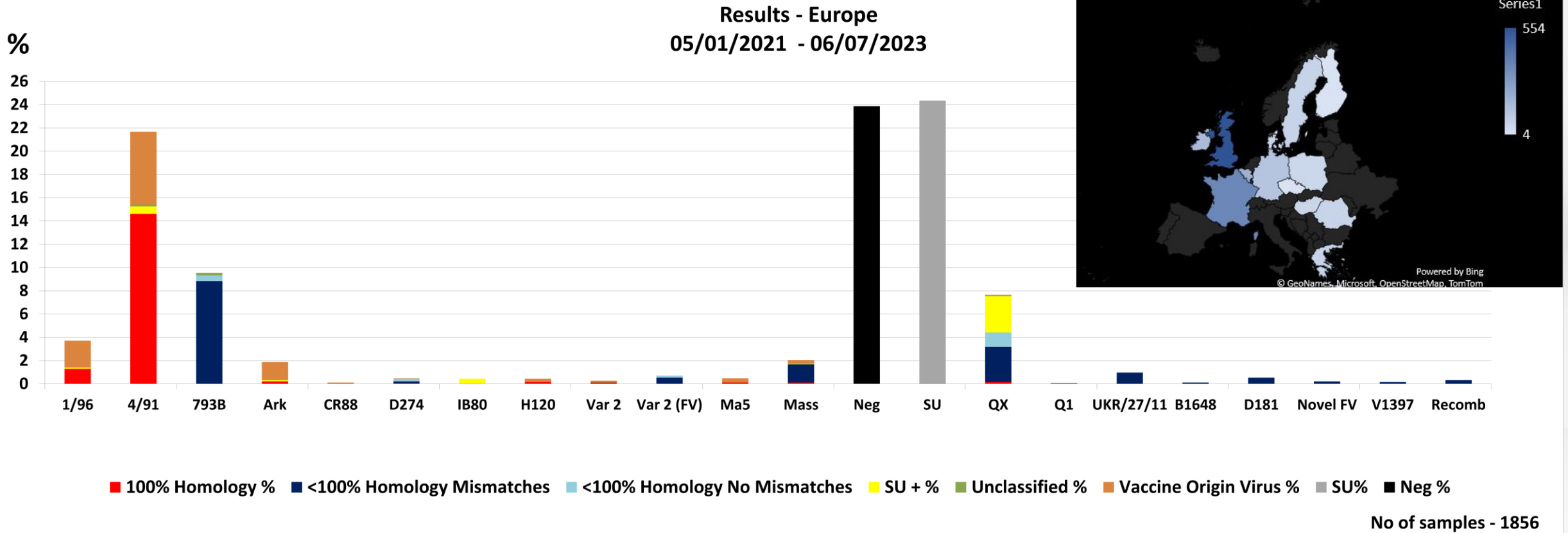
Nucleic Acid Sequence	99% Homologous to IBV/SULI2/2017 reference strain		
Position	Vaccine Vial Sequence	Sample Sequence	Comments

FV arrived in advance of the onset of immunity (6 dpv)

A clear problem with the application of Ma5 component of the vaccination programme

Numerous Diverse Genotypes of IB Field Viruses Circulate Around Europe and CER

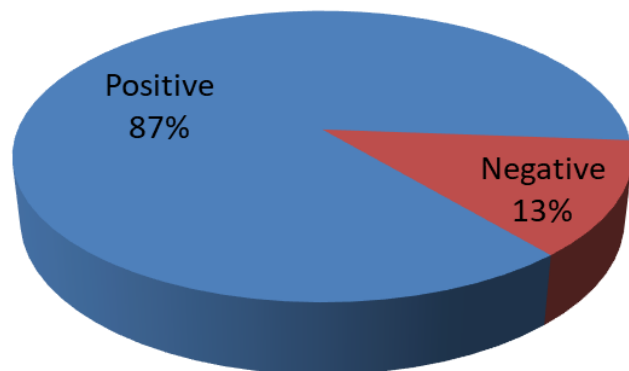
EUROPE (2021-2023)



Total number of flocks evaluated: 1744 (1856 samples collected)
 Novel Fvs (4); Recombinant (6)

CER REGION (2018-2024)

Results - PL-CZ-HU-RO
05/01/2018 - 03/05/2024



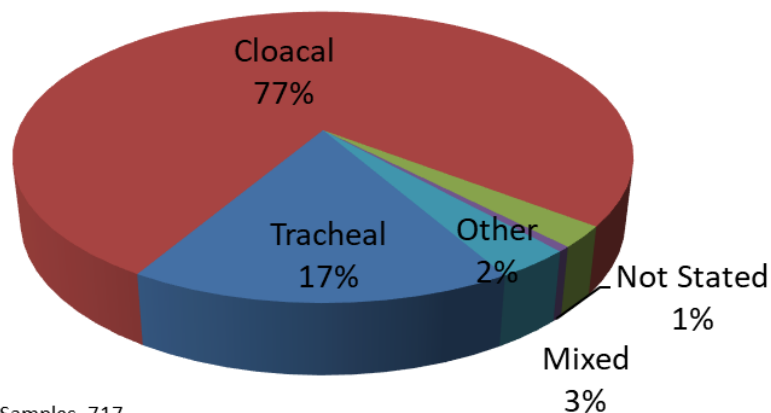
No of samples - 717

N. submissions: 664
N. Samples tested: 717

Country	Submission
C. Republic	20
Hungary	99
Poland	420
Romania	125
total	664

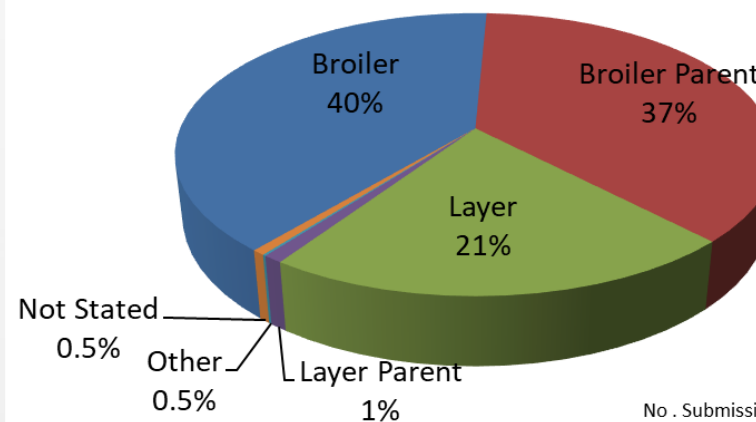


Results - PL-CZ-HU-RO
05/01/2018 - 03/05/2024



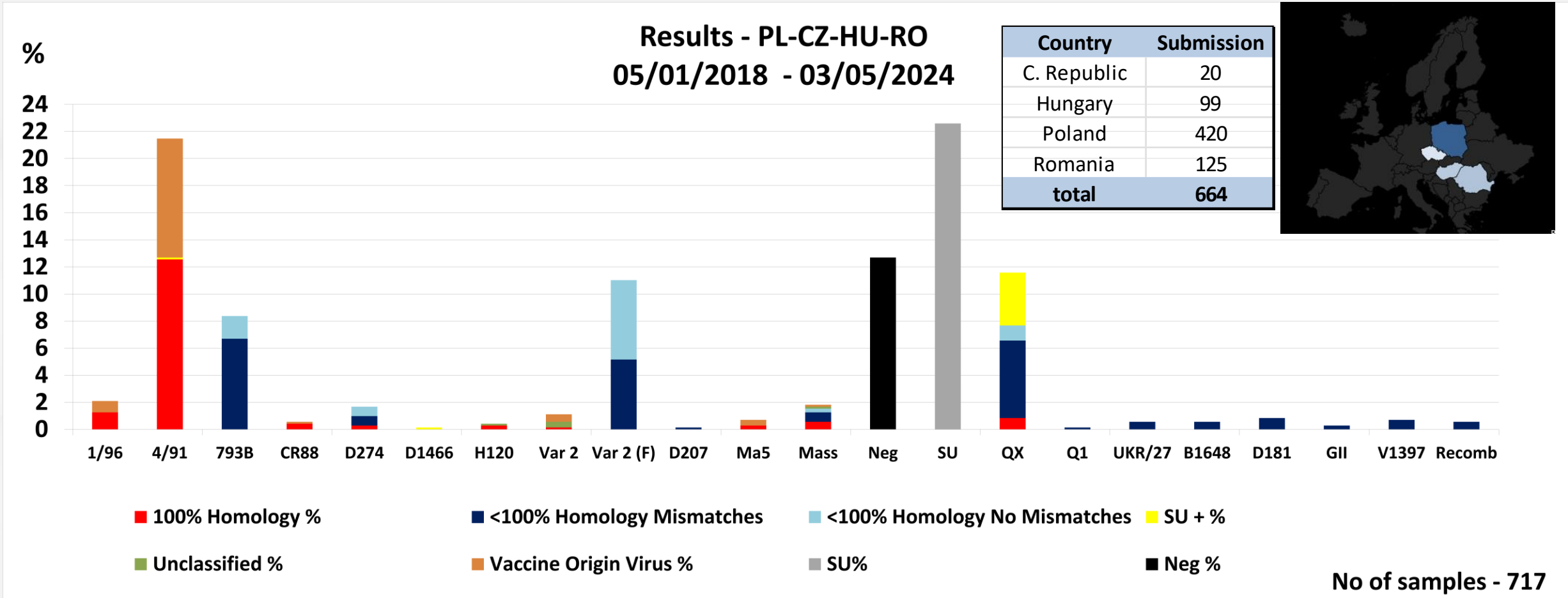
No of Samples- 717

Results - PL-CZ-HU-RO
05/01/2018 - 03/05/2024



No. Submissions (flocks) - 664

CER REGION (2018-2024)



SU (untypeable strains): Samples that didn't react to any sequencing protocol (mainly due to small quantity of RNA present)

SU +: Samples that didn't react to any sequencing protocol but tested positive to a specific PCR (Mass, 4/91, 1/96, QX)

Unclassified: Strains that was not possible to classify given the poor quality of the sequence

Recombinant (4): Mass-793B (2); 793B-QX (1); CK/CH/YN/SL12-1-793B (1)

GII: CK/PL/G067/2015 (GII)

Long Lived Birds (2018-2024)- 793B

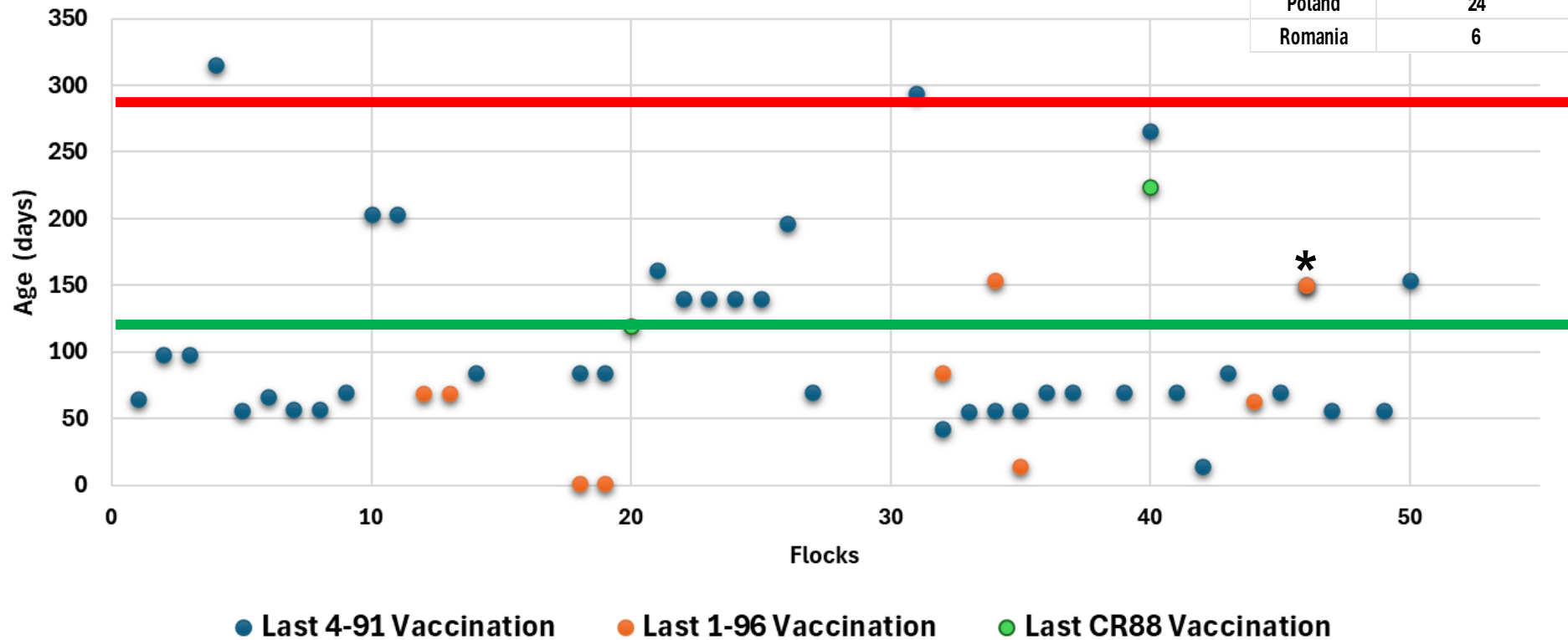
Vaccines used in 793B-infected flocks (N=50)

FEW FLOCKS USING IN LAY VACCINATION



793B FV In Long Lived Birds Flocks (N= 50)
Last 793B Vaccine Application

Country	Cases
Czech Republic	5
Hungary	15
Poland	24
Romania	6



The red line represents the average age when the field challenge was recovered (287 days or 41 weeks). The green line represents the beginning of the laying period (≈ 126 days). * 4/91 and 1/96 genotype vaccines used repeatedly in lay.

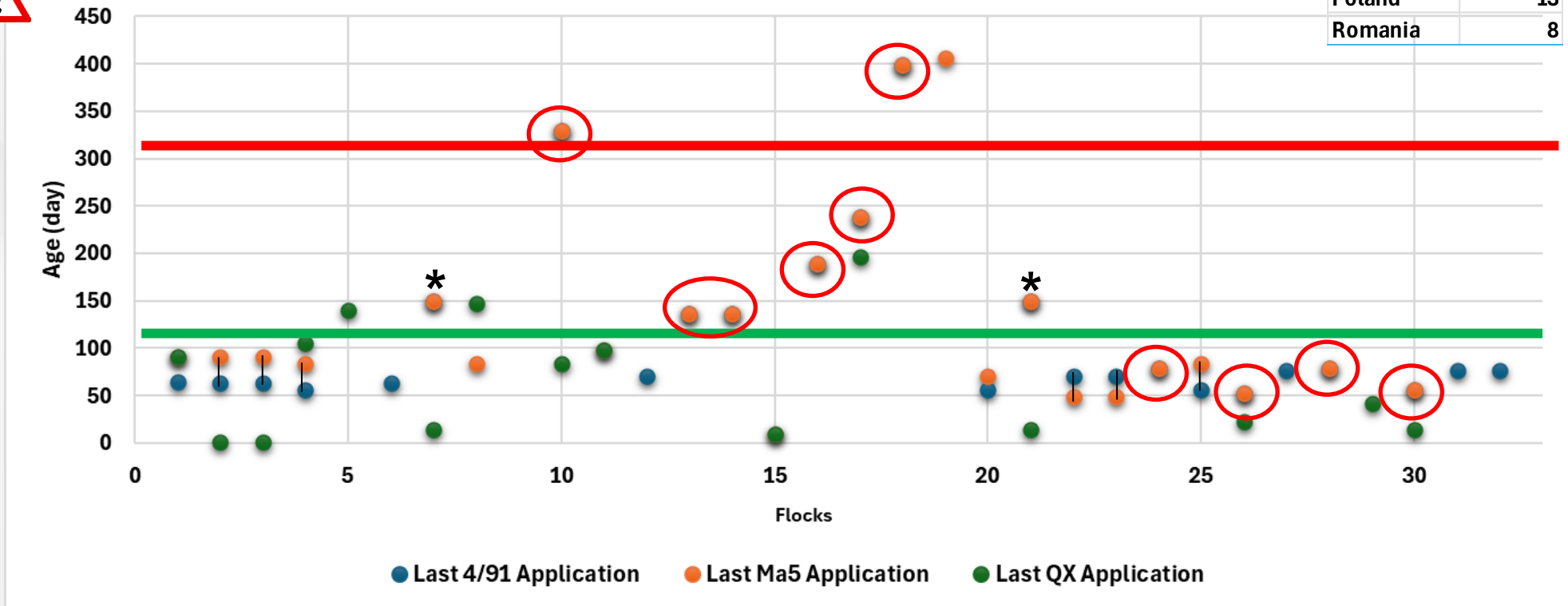
Long Lived Birds (2018-2024) - Vaccines used in QX-infected flocks (N=32)

FEW FLOCKS USING IN LAY VACCINATION



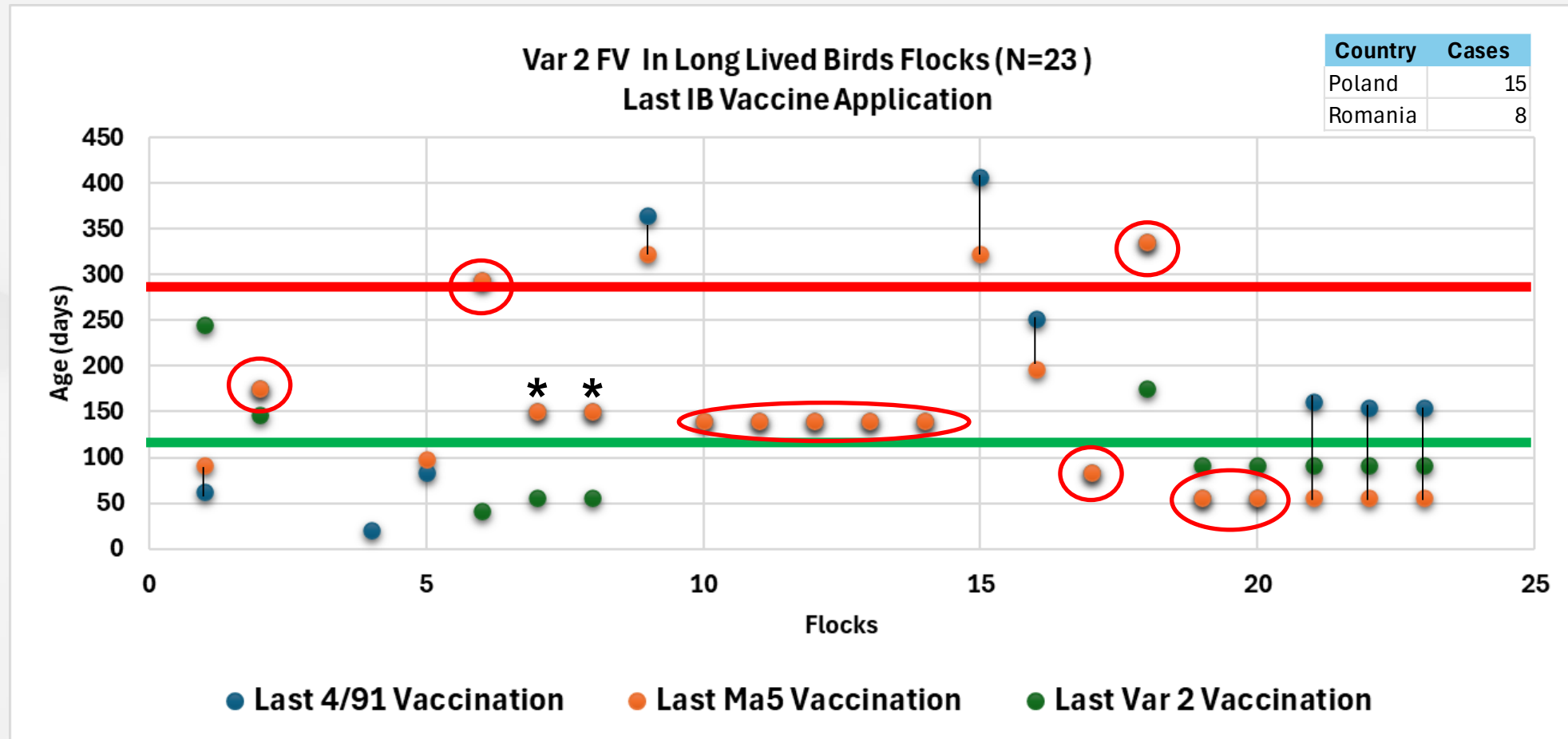
QX FV In Long Lived Birds Flocks (N= 32)
Last IB Vaccine Application

Country	Cases
Czech Rep	4
Hungary	7
Poland	13
Romania	8



The red line represents the average age when the field challenge occurred (308 days or 44 weeks). The green line represents the beginning of the laying period (≈ 126 days); *Used repeatedly in lay; Red circle: 4/91 and Ma5 genotype vaccines applied together.

Long Lived Birds (2018-2024)- IB Vaccines used in Var 2-infected flocks (N=23)

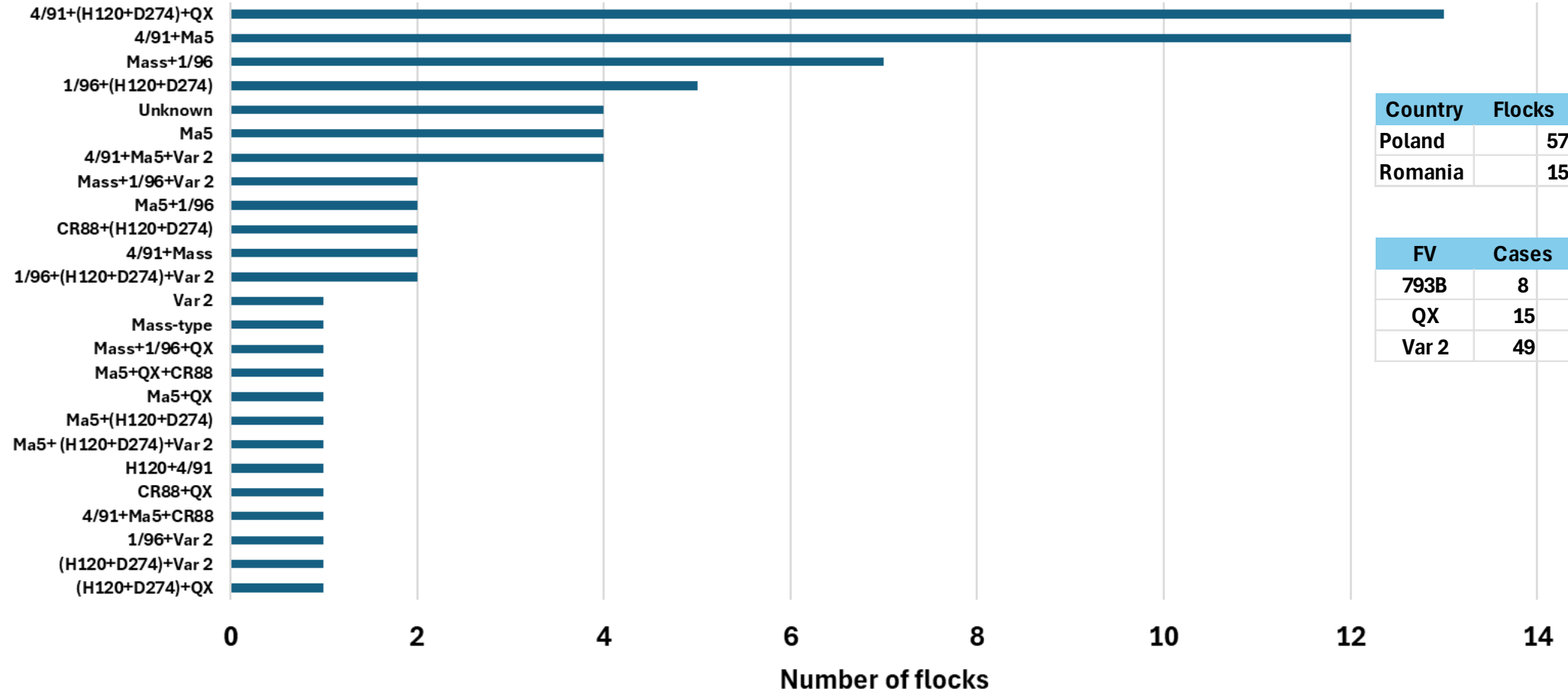


The red line represents the average age when the field challenge occurred (292 days or 41 weeks). The green line represents the beginning of the laying period (≈ 126 days); *Used repeatedly in lay; Red circle: 4/91 and Ma5 genotype vaccines applied together.

Broilers (2018-2024)- IB Vaccines used in infected flocks (N=72)



IB Vaccination Programs Used in Infected Broiler Flocks (N=72)





Opportunities for Vaccination Program Modification

- Choosing adequate vaccination program - evaluation of vaccine take
- Ensuring the timing of onset of immunity following vaccination
- Too large a gap between protectotype vaccines - close the gap
- Live vaccination only in the rearing period - extend into lay
- Insufficient intensity of protectotype vaccination in lay - increase administration frequency

- **Molecular testing of infectious bronchitis viruses**
 - creates technical opportunities to improve production
 - Important tool to evaluate the circulation of IB strains
- **Big datasets demonstrate the association of suboptimal vaccination programs with increased recoveries of the major global field viruses (793B, QX and Var 2) creating a clear opportunity to improve clinical outcomes.**
- **Application of the ProtectoTake test and IB sequencing in the framework of the diagnostic algorithm supports veterinarians with a practical vaccine application QA/QC program.**

THANK YOU!

X-VO

