THE UNIVERSITY OF GEORGIA
College of Veterinary Medicine

Avian *Mycoplasma*

Naola Ferguson-Noel, DVM, MAM, PhD

If Georgia were a country, it would be the 7th largest in Broiler Production

(1,000 metric tons forecast for 2014)

United States	17,276
China	12,700
Brazil	12,678
India	3,725
Russia	3,100
Mexico	3,060
GEORGIA	2,484
Argentina	2,080
Turkey	1,810
Indonesia	1,600

Prepared by: Georgia Poultry Federation
Source: USDA/FAS
Updated: July 2014

ON AN AVERAGE DAY GEORGIA PRODUCES

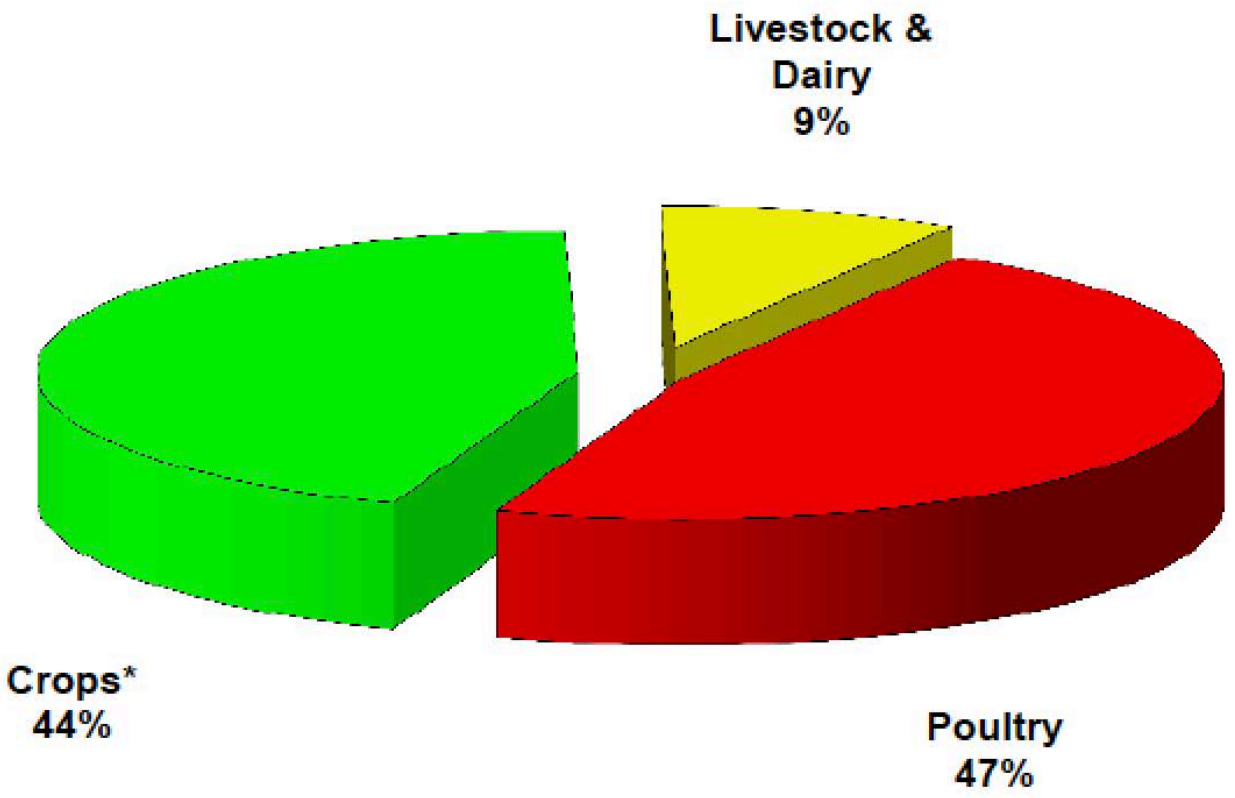
29.3 ***MILLION POUNDS OF CHICKEN***

6.9 ***MILLION TABLE EGGS***

5.5 ***MILLION HATCHING EGGS***

Prepared by: Georgia Poultry Federation
Source: USDA NASS
Updated: July 2014

Poultry -- The Largest Segment of Georgia Agriculture



Percent Total by Commodity

* Crops include row crops, vegetables, fruits, nuts, nursery/greenhouse, hay and turfgrass

Prepared by: Georgia Poultry Federation
Source: University of Georgia, 2011 Farm Gate Value Report
Updated: July 2014

Production of poultry meat and eggs, leading nations, 2001

In metric t ons

Nation	Poultry meat	Chicken meat	Turkey meat	Duck meat	Goose meat	Primary eggs*
WORLD	**70,358,813**	**60,258,645**	**5,085,889**	**2,936,687**	**2,058,969**	**56,594,078**
United States	16,747,600	14,210,000	2,485,000	52,600	Neg.	5,080,000
China	13,286,850	9,401,030	1,990	2,009,980	1,873,850	23,354,520
European Union (15)	8,852,099	6,632,852	1,860,960	343,112	14,075	5,303,441
Brazil	6,394,850	6,222,700	165,000	7,150	Neg.	1,582,700
France	2,077,100	1,100,000	735,000	235,000	6,000	1,047,000
Mexico	1,945,038	1,897,546	27,242	20,250	Neg.	1,881,645
United Kingdom	1,561,700	1,257,500	256,000	45,800	2,400	644,751
Thailand	1,366,500	1,260,000	Neg.	105,000	1,500	810,000
Japan	1,180,012	1,180,000	12	Neg.	Neg.	2,526,000
Italy	1,156,000	816,000	340,000	Neg.	Neg.	707,000
Canada	1,092,300	943,000	141,000	7,400	900	362,800
Spain	1,034,000	1,012,000	22,000	Neg.	Neg.	563,700

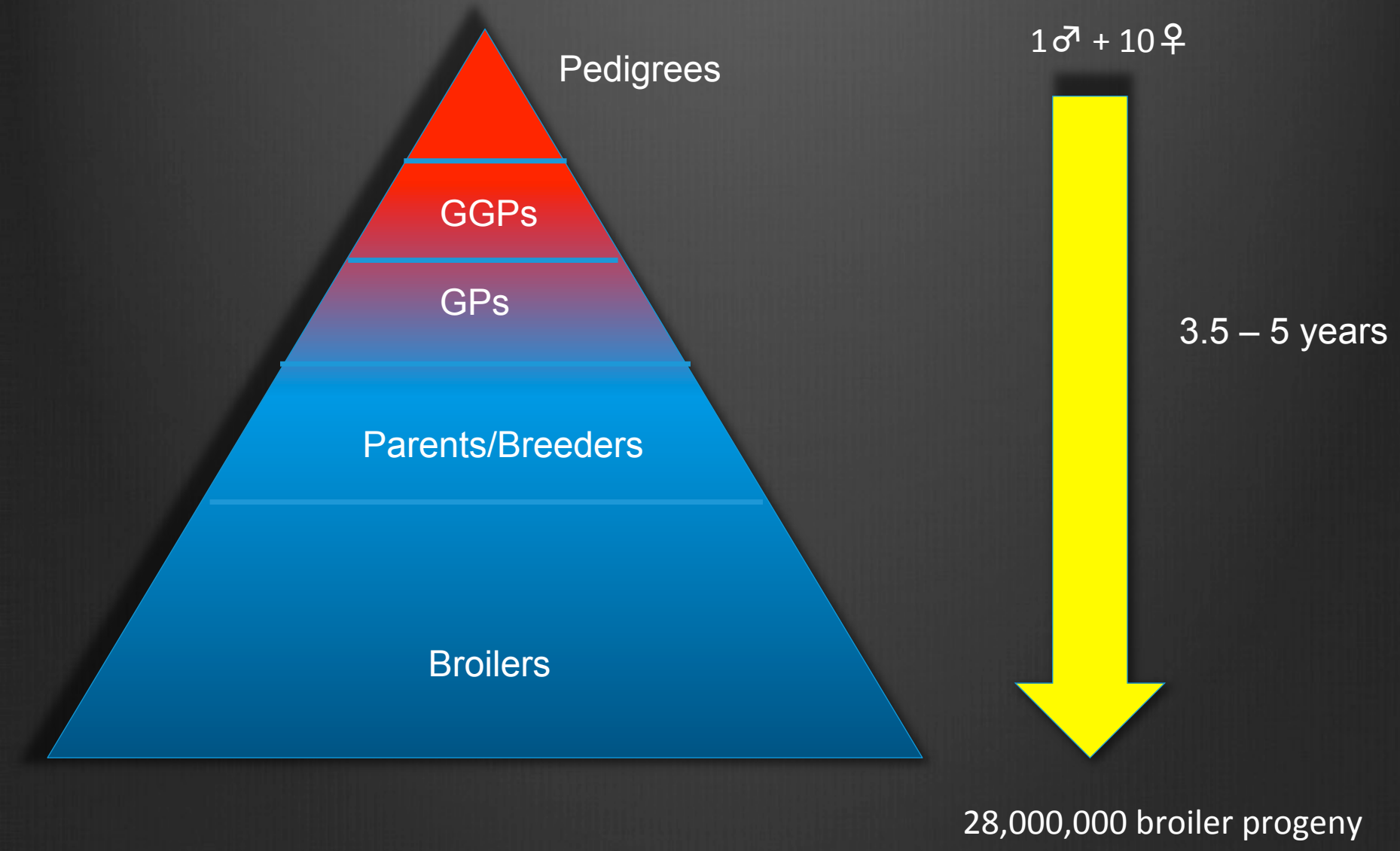
*Table eggs of all species, including chicken, duck, and goose.

Neg.: Negligible production


2006/11/25

http://poultryhealthtoday.com/poultry-veterinarians-the-latest-focus-of-popular-youtube-series/?utm_source=PHT+eBlast+List&utm_campaign=ab73f81402-Samantha_Pohl_announcement8_20_2015&utm_medium=email&utm_term=0_5ac605299a-ab73f81402-261557785

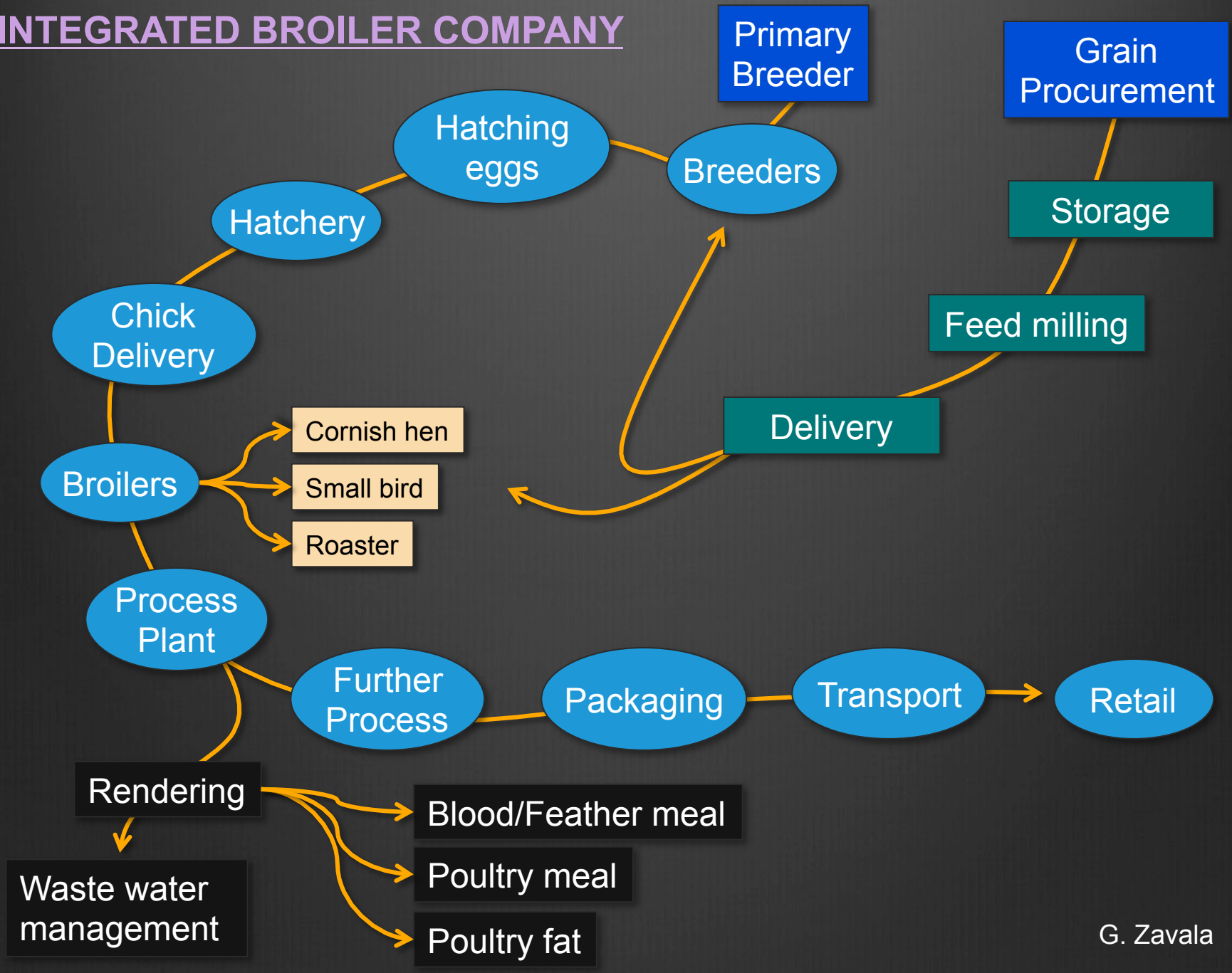
https://www.farmaid.org/issues/industrial-agriculture/a-look-at-the-poultry-industry-how-does-chicken-get-on-your-plate/


Pedigrees
GGPs
GPs
Parents/Breeders
Broilers
1♂ + 10♀
3.5 – 5 years
28,000,000 broiler progeny

Output of a Pedigree Family

- Pedigree family = 1♂ + 10♀
- Pedigree to broiler = 3.5 - 5 years
- Up to 28,000,000 broiler progeny
- (28KK) x (2.0 Kg) x (69% yield):
  - 39,744 T of poultry meat

INTEGRATED BROILER COMPANY



Structure of a Broiler Integration

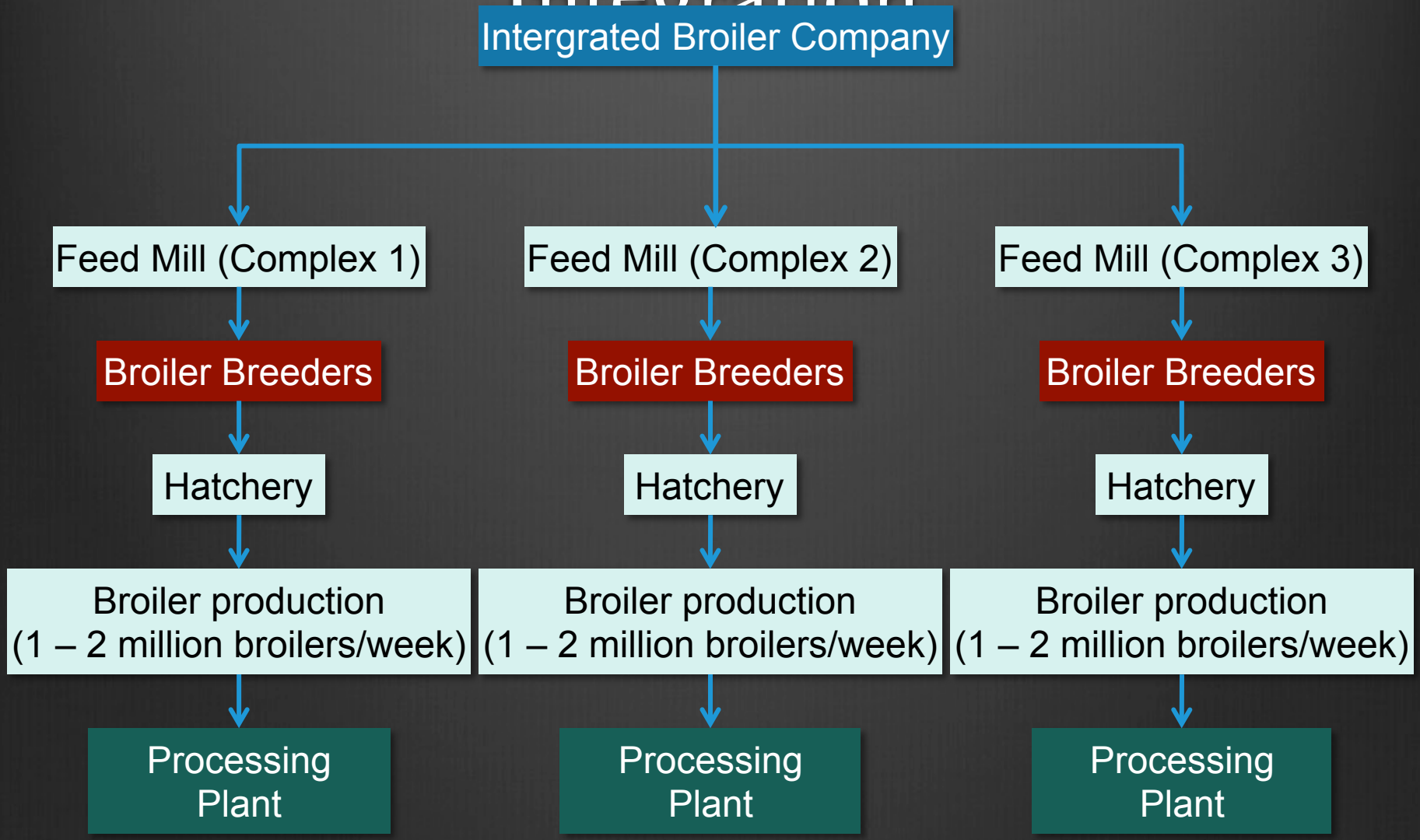
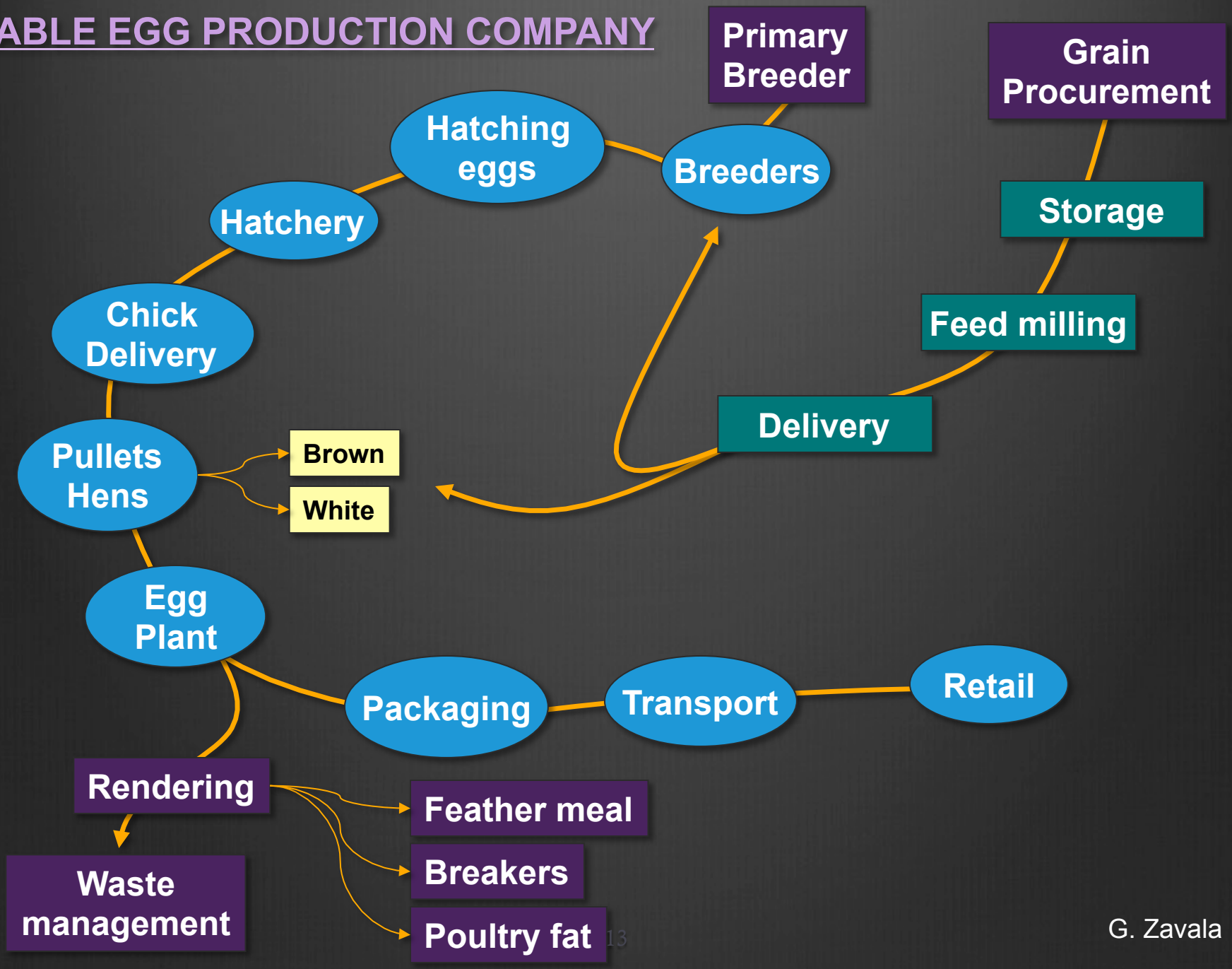
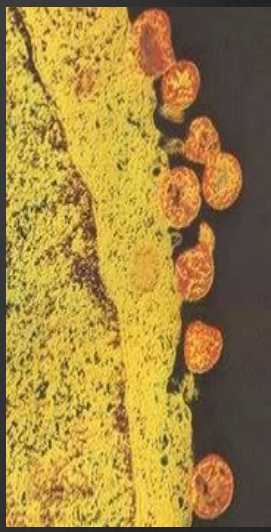


TABLE EGG PRODUCTION COMPANY



G. Zavala

Mycoplasma gallisepticum and M.synoviae



- Bacteria
  - Mollicutes
    - No cell wall
    - Smallest free-living organisms
    - Smallest genome of any free-living organism

- pathogenic and economically significant avian mycoplasma

Why is Control Important?…

- Clinical disease – pathogenic strains

The World Organisation for Animal Health (OIE)

- OIE-Listed diseases
- Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

NPIP

- National Poultry Improvement Plan (NPIP)
  - cooperative Federal-State-Industry program
  - use new diagnostic technology to effectively improve poultry and poultry products
  - egg-transmitted, hatchery-disseminated poultry diseases
  - provides certification that poultry and poultry products are disease free
  - **Pullorum-Typhoid (P-T)**
  - **Mycoplasma gallisepticum (MG)**
  - **Mycoplasma Synoviae**
  - **Mycoplasma Meleagridis**
  - **Salmonella Enteritidis**
  - **Avian Influenza (AI)**

Figure 7

NPIP

The official tests for *M. gallisepticum, M. meleagridis,* and *M. synoviae* shall be

- the serum plate agglutination test,
- the tube agglutination test,
- the hemagglutination inhibition (HI) test,
- the microhemagglutination inhibition test,
- the enzyme-linked immunosorbent assay (ELISA) test,[3]
- a polymerase chain reaction (PCR)-based test, or a combination of two or more of these tests.

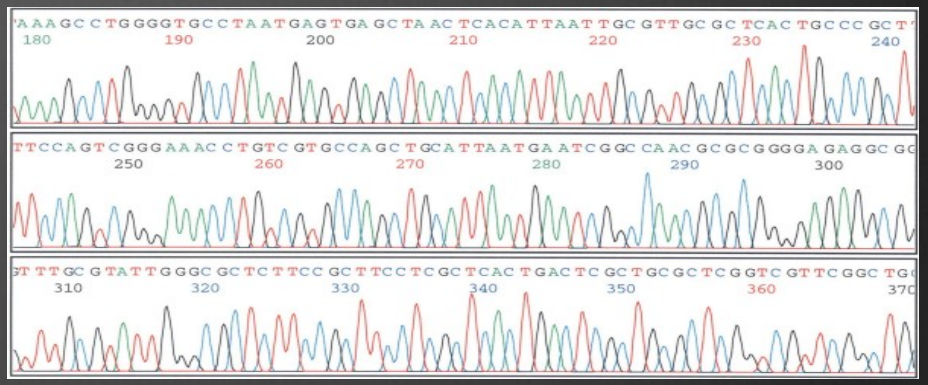
The HI test or the microhemagglutination inhibition test shall be used to confirm the positive results of other serological tests. HI titers of 1:40 or more may be interpreted as suspicious, and final judgment must be based on further samplings and/or culture of reactors.

Avian Mycoplasma Diagnosis

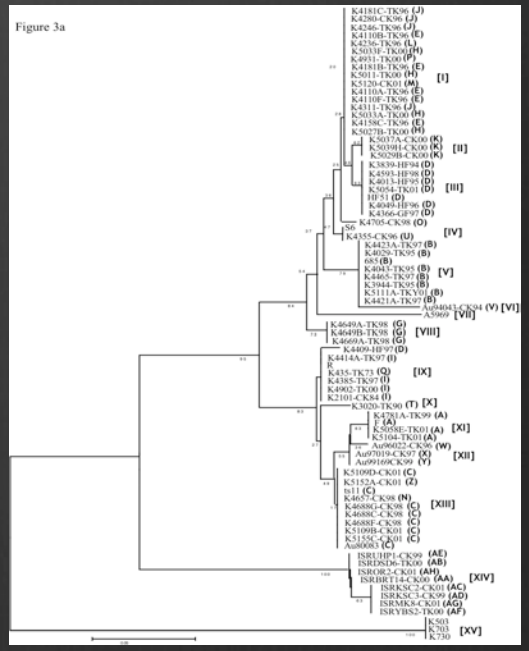
- Serology - SPA, HI and ELISA
- PCR – conventional and real-time
- Culture
- Bioassay

Gene Targeted Sequencing (GTS)

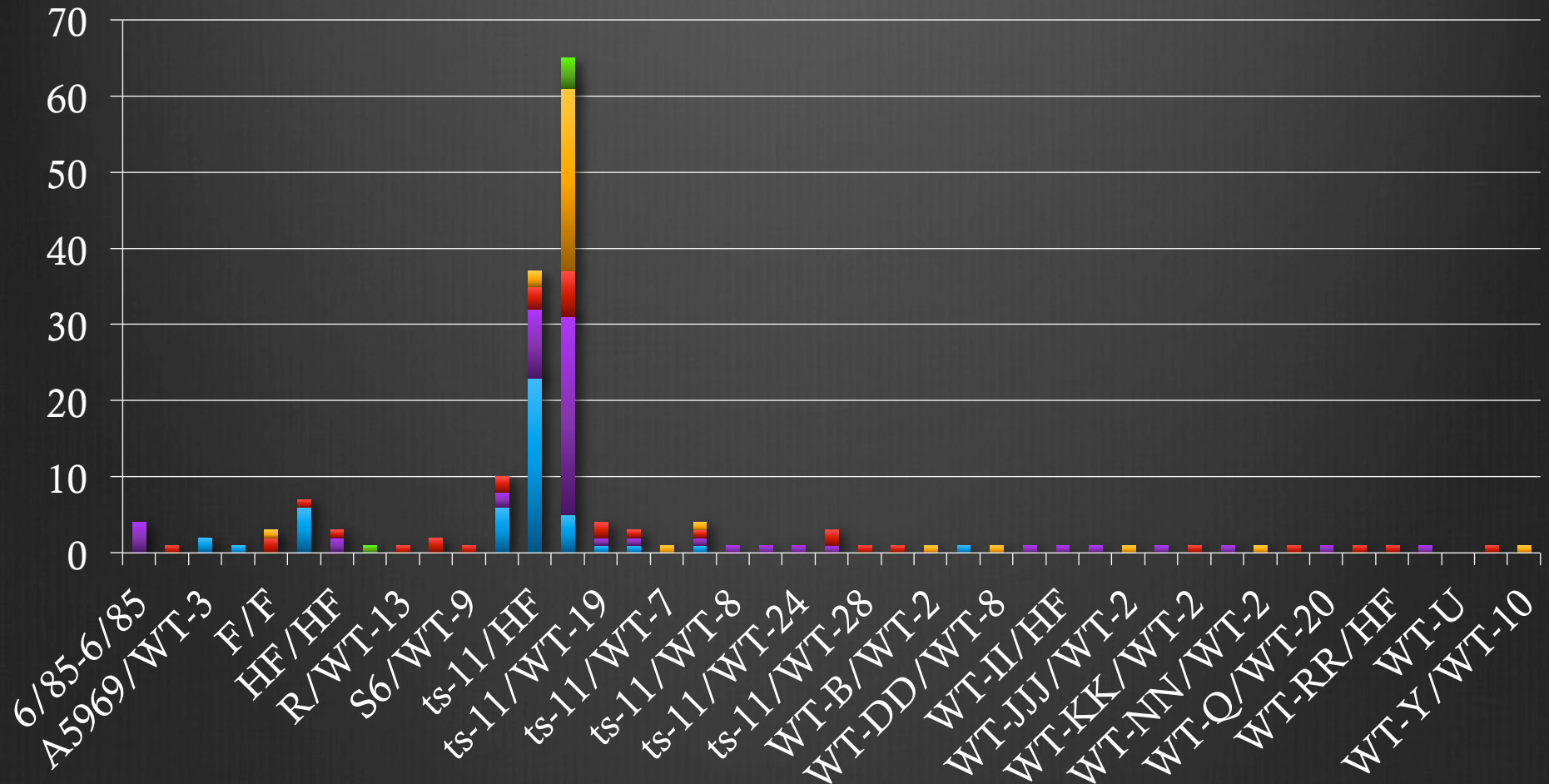
- Compare to database
  - >200 sequences
- Assign type
  - WT#BB/WT#4
  - S-1, S-2, S-3



- No culture required
- Good reproducibility
- Reference database
- Can be combined with diagnostic PCR



MG Types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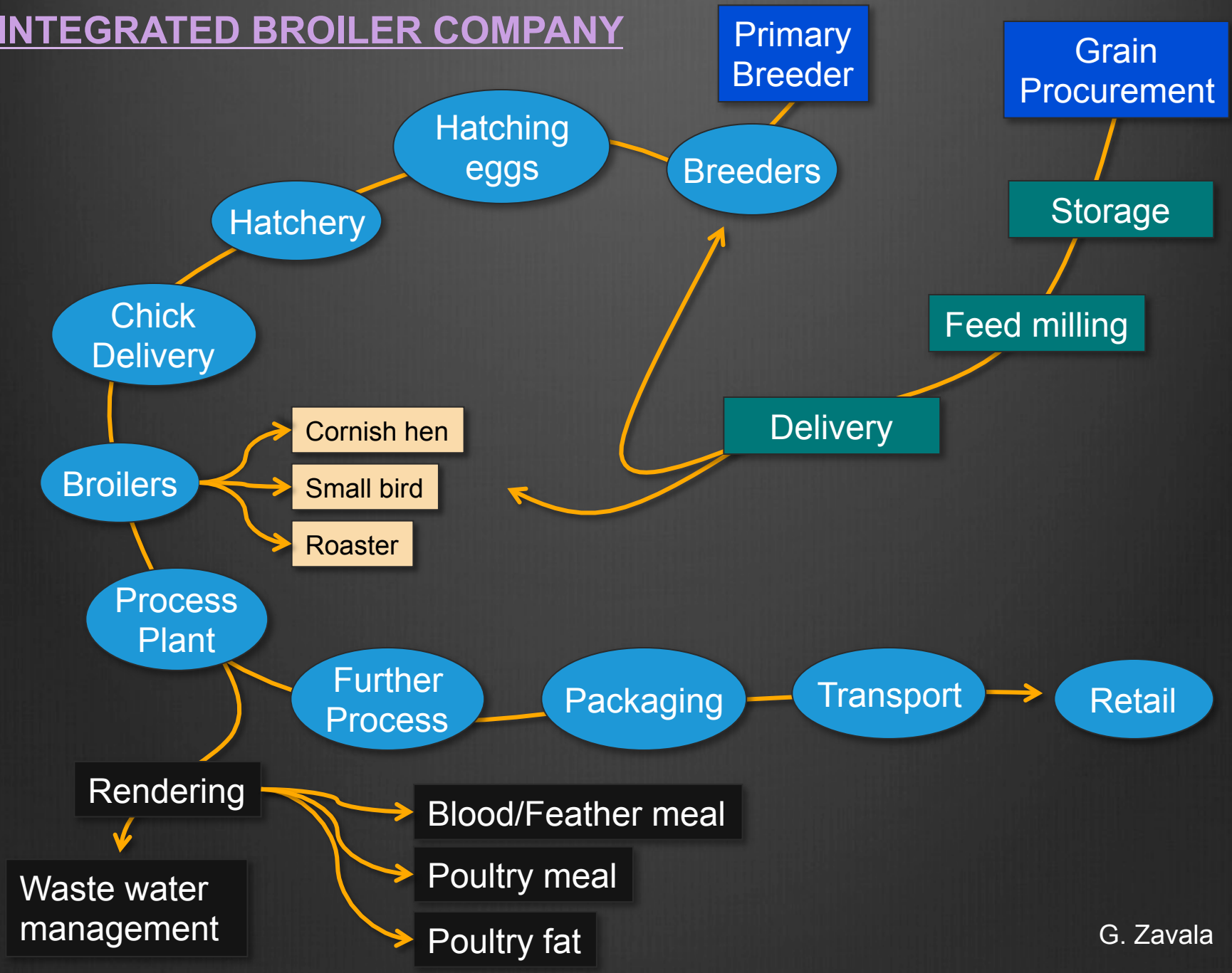
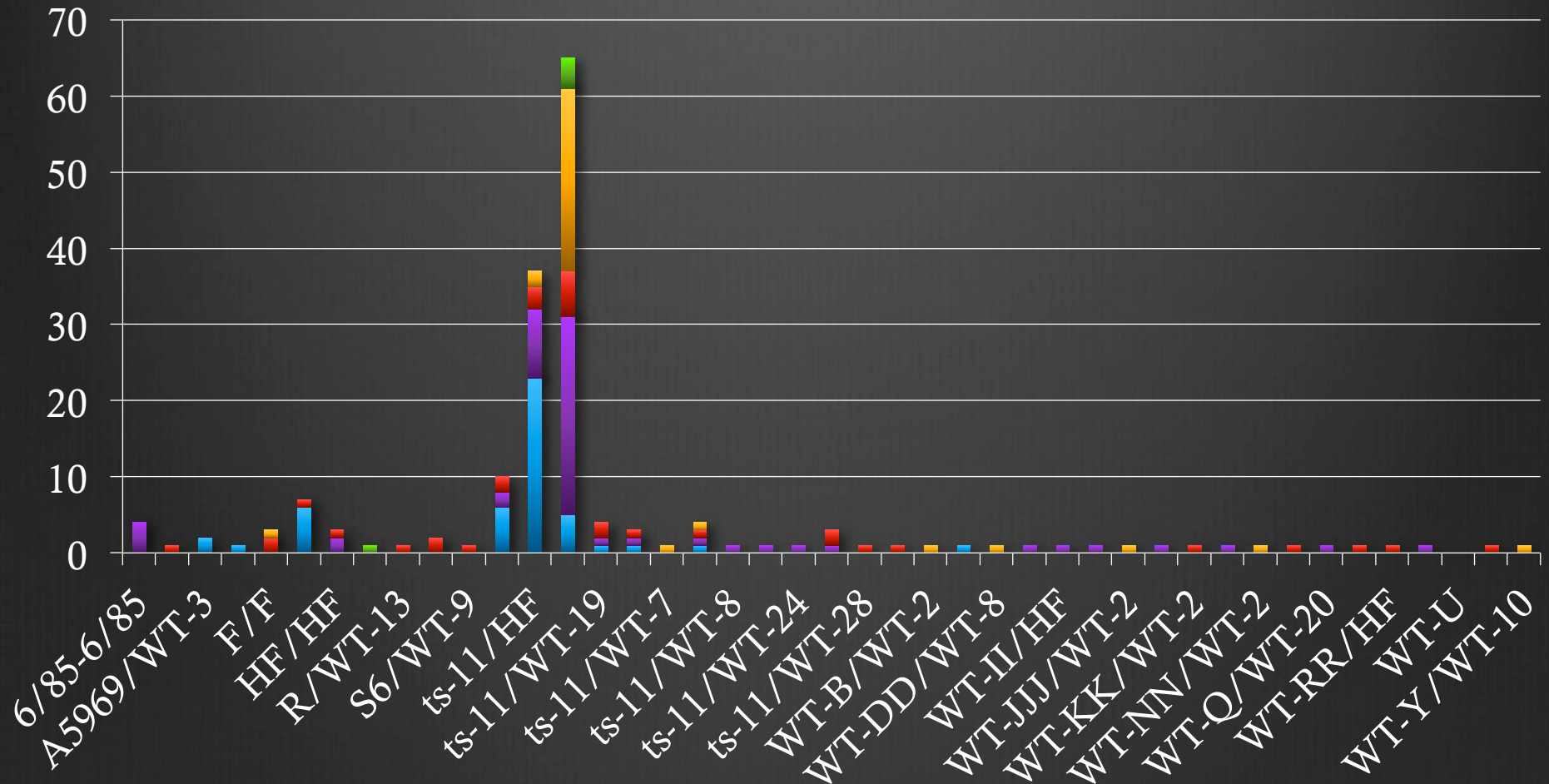
HF/HF
R/WT-13
S6/WT-9
ts-11/HF
ts-11/WT-19
ts-11/WT-7
ts-11/WT-8
ts-11/WT-24
ts-11/WT-28
WT-B/WT-2
WT-DD/WT-8
WT-II/HF
WT-JJJ/WT-2
WT-KK/WT-2
WT-NN/WT-2
WT-Q/WT-20
WT-RR/HF
WT-U
WT-Y/WT-10

INTEGRATED BROILER COMPANY



MG Types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WT-NN/WT-2
WT-Q/WT-20
WT-RR/HF
WT-U
WT-Y/WT-10

Approaches to Control

- Keep it out
  - Surveillance
  - Eradication
- Live with it
  - Medication
  - Vaccines

Mycoplasma Surveillance Tests

- Sensitive
  - early detection
- High throughput
- Economical
- Tolerate some false positives
- SPA, ELISA, (real-time PCR)

Sources of Infection

- Egg transmission
  - Rate of transmission unpredictable

Vertical transmission

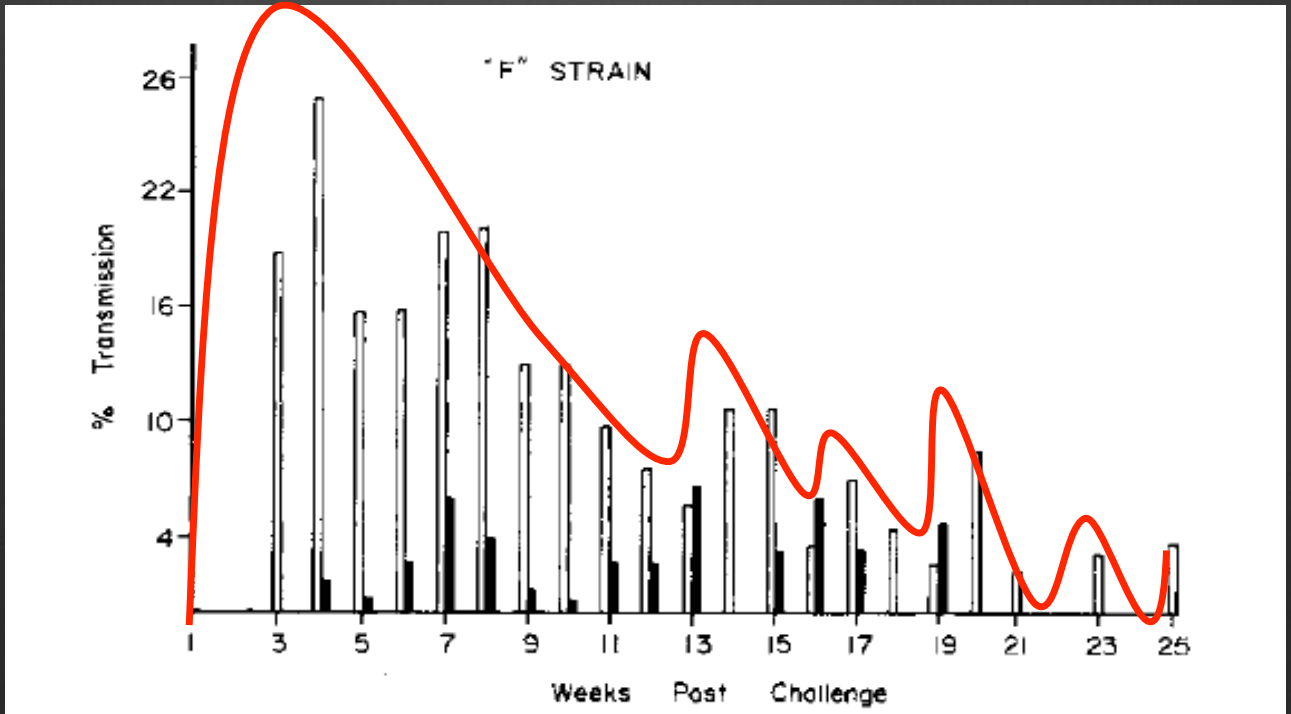


Fig. 1. Egg transmission of F-strain-vaccinated chickens (black bar) and unvaccinated controls (white bar).

Glisson, J.R. & Kleven., S.H. (1984). *Avian Dis,* 28, 406-415.

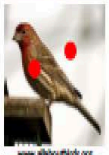
Sources of Infection

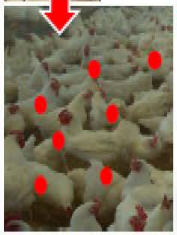
- Egg transmission
  - Rate of transmission unpredictable
- Horizontal transmission
  - Direct or indirect contact with infected birds
  - Biological carriers
  - Mechanical carriers – dust, droplets, feathers, equipment, rodents
  - Aerosol transmission possible over short distances

Mycoplasma on GA farms: Prevention and Containment

Mycoplasma infections (MG or MS) are important because positive flocks can transmit the organism to the progeny (usually broilers), possibly making them sick. Mycoplasma positive breeder flocks may also show signs of disease, affecting their productivity. Hatching eggs exports are disturbed. The flocks lose their "clean" NPIP classification and may have to be sold early.

● : MG or MS





My birds tested positive: how did they get it?

THE MOST COMMON WAY TO GET MYCOPLASMA IN THE FIELD: IT WALKS IN, USUALLY ON TWO LEGS!

DIRECT CONTACT OF THE FARM OR GROWER with:
Persons, vehicles, equipment that have been in contact with non-commercial poultry, commercial layers, positive farms: hired help, contractors, crews, family members, other visitors are by far the most common means of infecting a flock!

A FARM CAN ALSO GET MYCOPLASMA FROM MIGRATION (less likely) from a Mycoplasma positive source or farm through rodents or insects

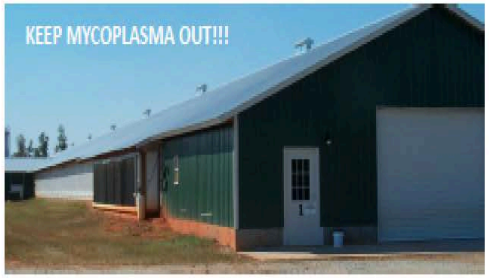
A FARM CAN ALSO GET CONTAMINATED THROUGH THE AIR or wild birds (least likely) MG and MS are sensitive to heat. They do not survive well outside the birds. However, they will withstand freezing. MG may also come from wild birds (ex. house finches)

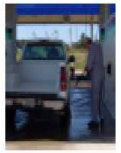
How do I keep Mycoplasma from spreading from my farm to others?

- Do not lend equipment out
- Practice enhanced rodent, insect control before load out.
- After load out, make sure all birds are disposed of promptly and properly.
- Keep house empty for a week before removing the litter; Wash and disinfect houses. (Mycoplasma are sensitive to disinfectants).
- In GA, MG infected birds have to be treated before transportation to decrease shed.

MOST IMPORTANTLY: How do I keep my birds CLEAN in the first place?

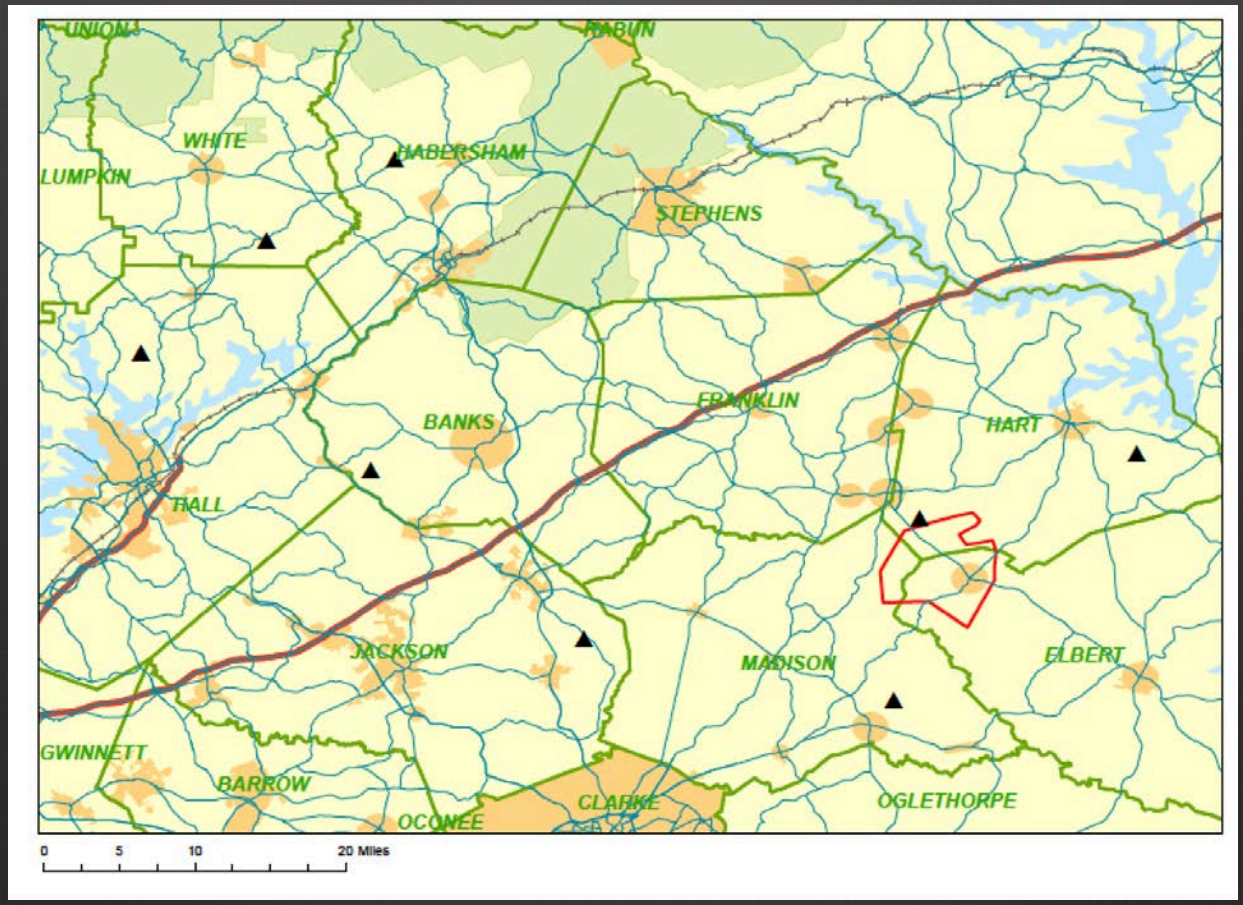
- Avoid contact with other birds
- Dispose of all dead birds promptly and properly
- Make sure your visitors and hired help have not been in contact with high risk poultry; No pets in houses
- Use dedicated equipment and vehicles on the farm
- Practice entry biosecurity at all times (no exceptions): Shower (on some farms), change of clothing or disposable coveralls, use dedicated footwear or disposable boots and hairnets, use foot pans.
  THIS IS VERY EFFECTIVE IN PREVENTING ENTRY!
- Bird proof houses, practice rodent and insect control





GPLN

February 2010
For educational purposes only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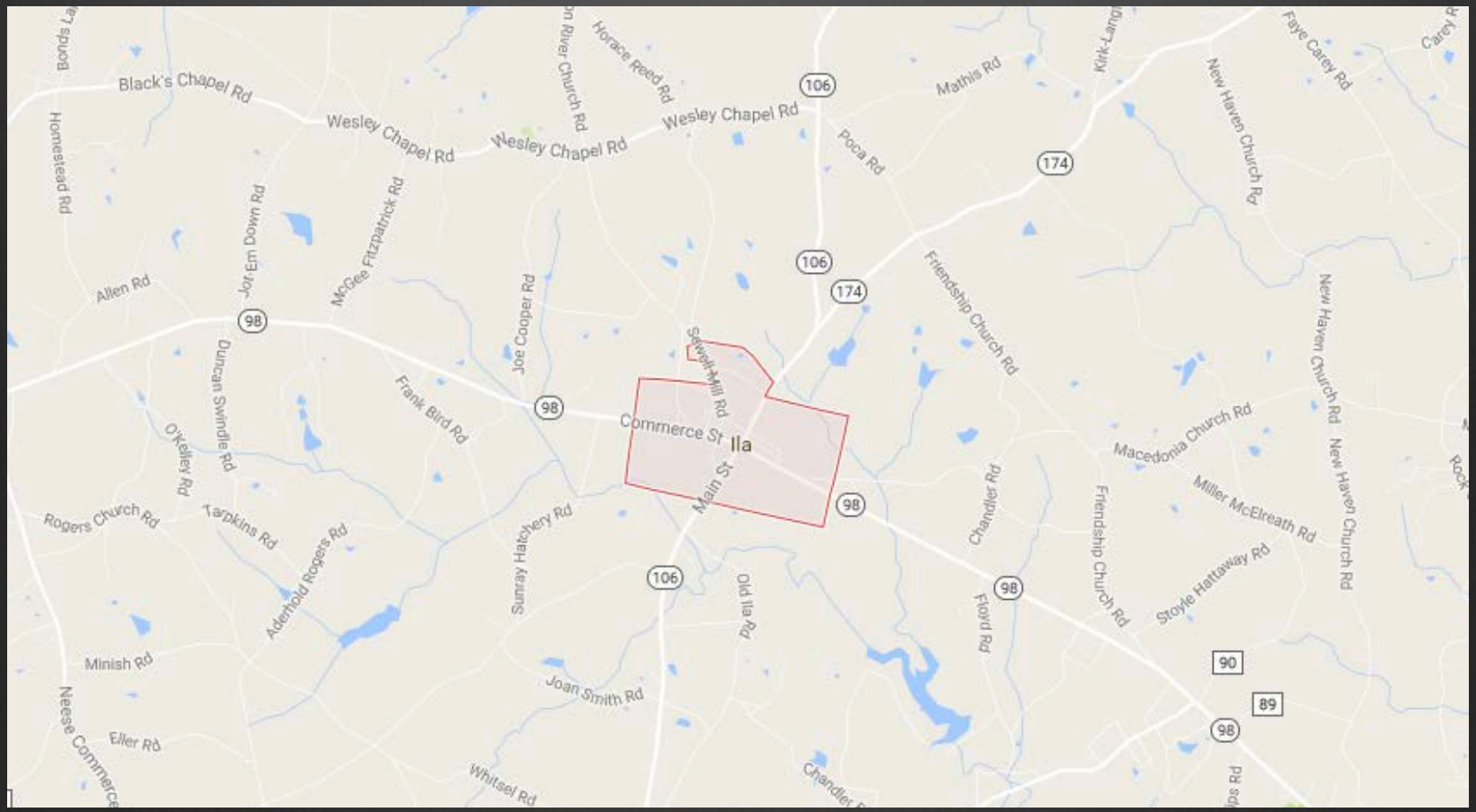
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Faye Carey Rd
New Haven Church Rd
Homestead Rd
Joe Em Down Rd
McGee Fitzpatrick Rd
Allen Rd
Joe Cooper Rd
Friendship Church Rd
Frank Bird Rd
Duncan Swindle Rd
O'Kelley Rd
Rogers Church Rd
Tarpkins Rd
Aderhold Rogers Rd
Sunray Hatchery Rd
Commerce St
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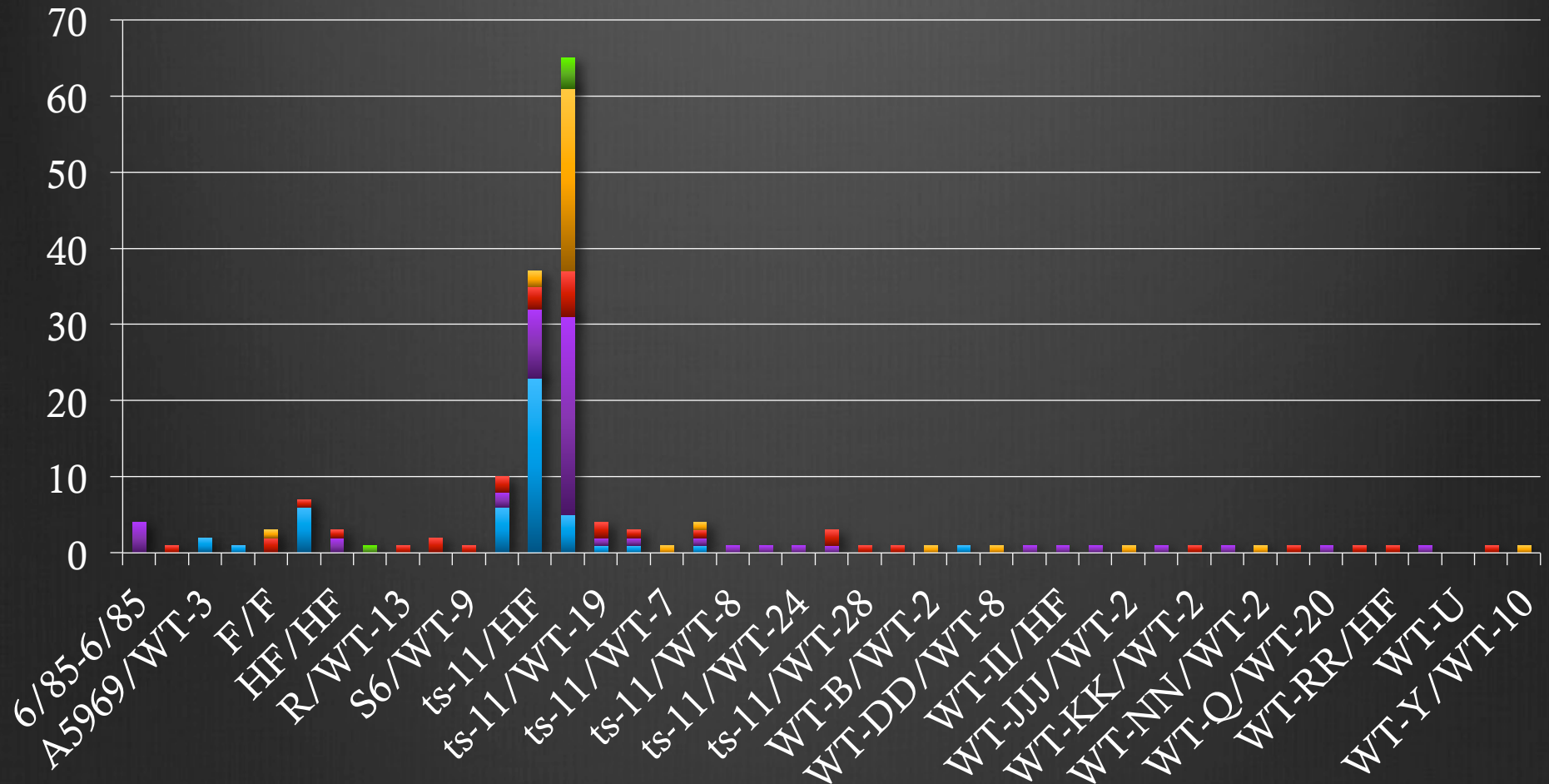
Main St
Old Ila Rd
Chandler Rd
Floyd Rd
Macedonia Church Rd
Miller McElreath Rd
Stoyle Hattaway Rd
Minish Rd
Joan Smith Rd
Eller Rd
Whitsel Rd
106
174
98
90
89

Survival of MG on Various Substances

Cotton	4 days	Feathers	4 days
Rubber	2 days	Hair	3 days
Straw	2 days	Ear	4 hours
Shavings	8 hours	Nose	1 day
Wood	1 day	Skin	< 4 hours
Feed	4 hours	Buffer	1 day

Christensen, N. H., C. A. Yavari, A. J. McBain, and J. M. Bradbury. Avian Pathol. 23:127-143. 1994.

MG Types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WT-NN/WT-2
WT-Q/WT-20
WT-RR/HF
WT-U
WT-Y/WT-10

Antibiotics

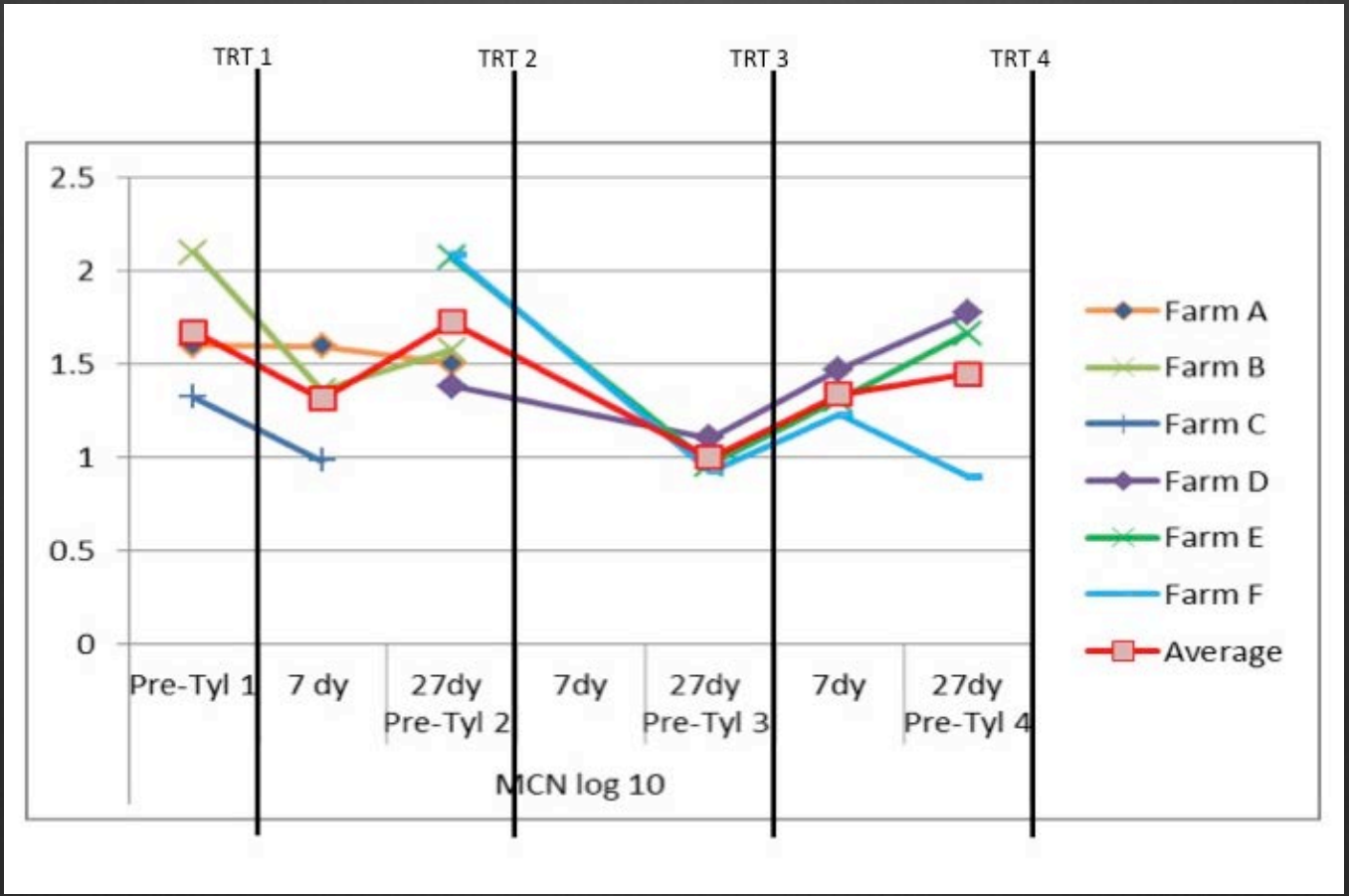
Antibiotics

- Will not eliminate infection
- Antibiotic resistance may develop

Genotype	Date Isolated	Company	MIC tylosin (µg/mL)	Sen		racycline /mL)	Sensitivity to tetracycline
S-56	Jun-12	1	1		S	.5	S
S-56	Jun-12	1	1		S	.5	S
S-48	Feb-13	2	0.0625		S	.5	S
S-56	May-14	5	2		I	25	S
S-56	May-14	5	1		S	.5	S
S-56	May-14	5	1		S	.5	S
S-56	Jun-14	5	1		S	25	S
S-56	Jun-14	4	1		S	.5	S
S-56	Jun-14	3	1		S	.5	S
S-56	Jun-14	3	1		S	.5	S
S-56	Jun-14	5	1		S	25	S
S-56	Jun-14	5	4		R	375	S
S-56	Jun-14	5	1		S	.5	S
S-56	Oct-14	5	1		S	25	S
S-56	Feb-15	2 (Farm A)	2		I	1	S
S-56	Feb-15	2 (Farm B)	1		S	1	S
S-56	Feb-15	2 (Farm C)	1		S	1	S
S-56	Mar-15	2 (Farm D)	2		I	.5	S
S-56	Mar-15	2 (Farm E)	4		R	2	S
S-56	Mar-15	2 (Farm F)	2		I	.5	S
			S ≤ 1 / I= 2 / R			≤ 4 / I= 8 / R > 16	

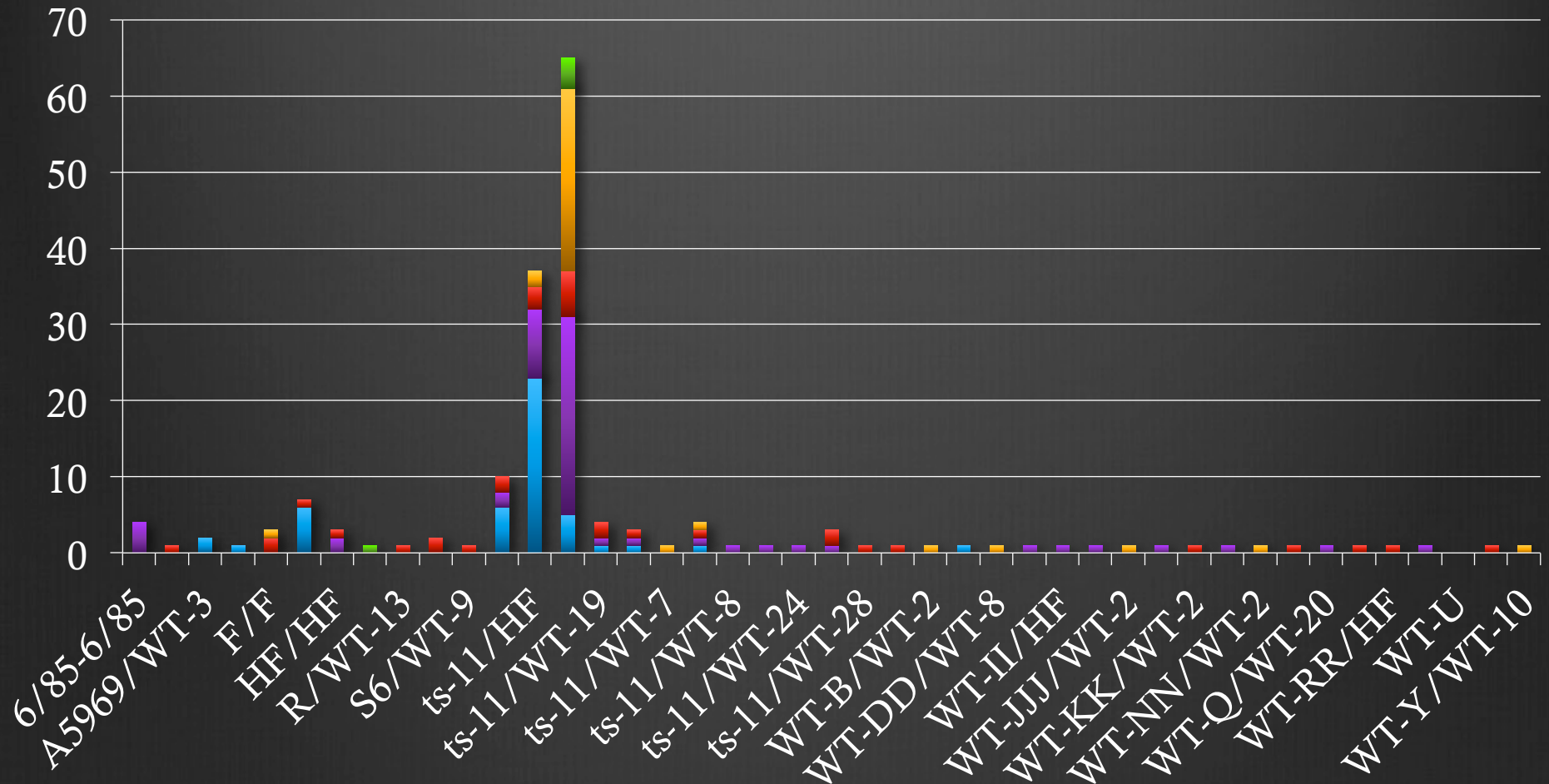
X. Gong & Ferguson-Noel, N., Unpublished (manuscript in preparation)

Tylosin Treatments Less Effective Over Time



A. Kiers & Ferguson-Noel, N., Unpublished (manuscript in preparation)

MG Types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WT-U
WT-Y/WT-10

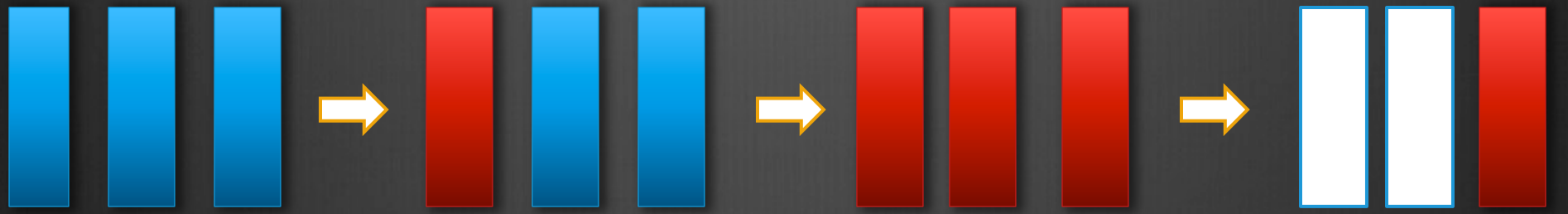
Vaccines

- Inactivated oil-emulsion bacterins
- Recombinant MG Vaccine
- Live vaccines
  - F Strain
  - ts-11
  - 6/85

Reasons to Vaccinate

- Prevent clinical disease
- Reduce egg transmission
- Avoid cost of medication
- Eradicate virulent field strains

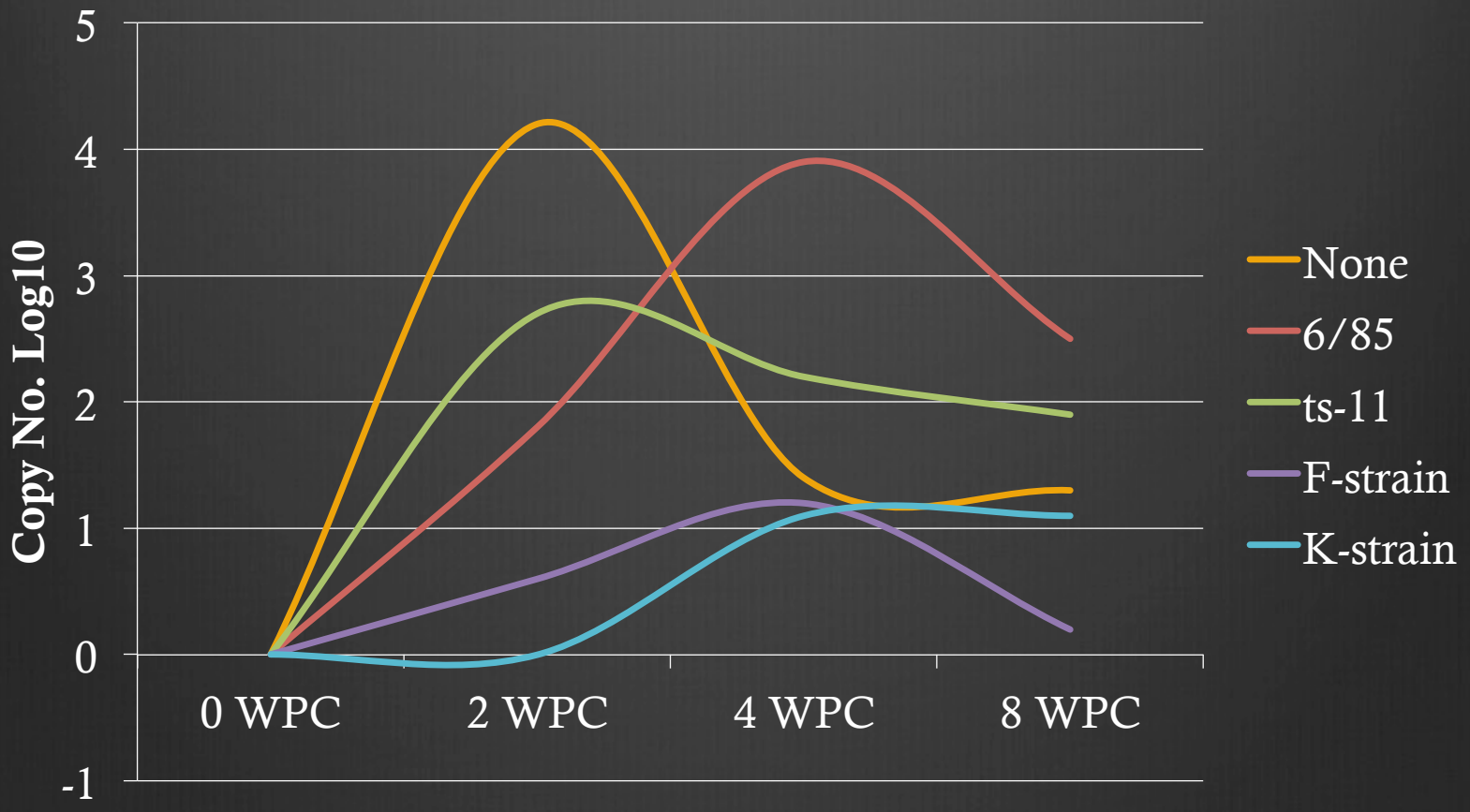
Displacement



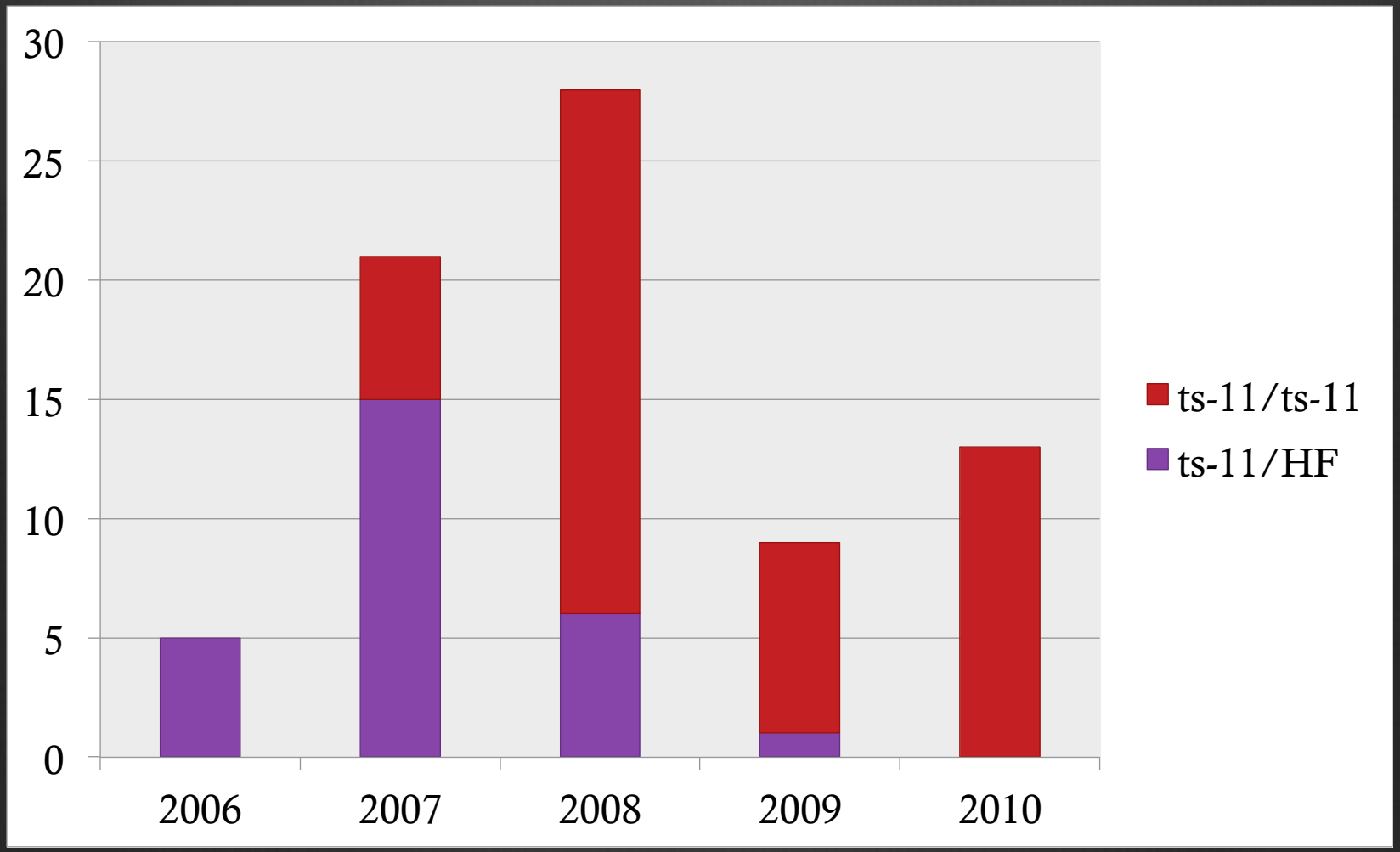
- Depends on ability of vaccine to prevent secondary infection with wild type

Wild type Vaccinated Clean

Displacement - R-strain experiment



MG – GA Broiler-type



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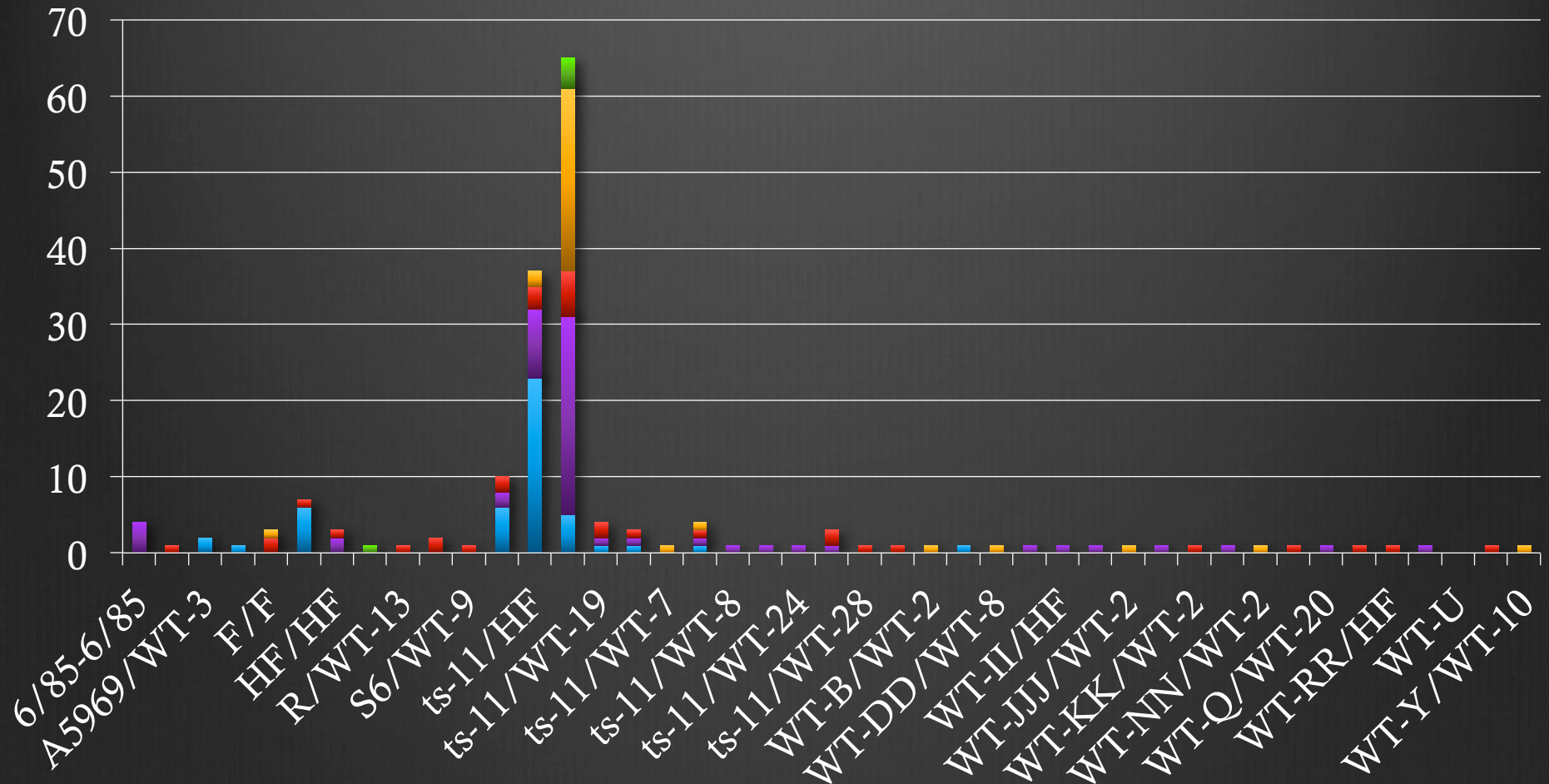
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WT-JJJ/WT-2
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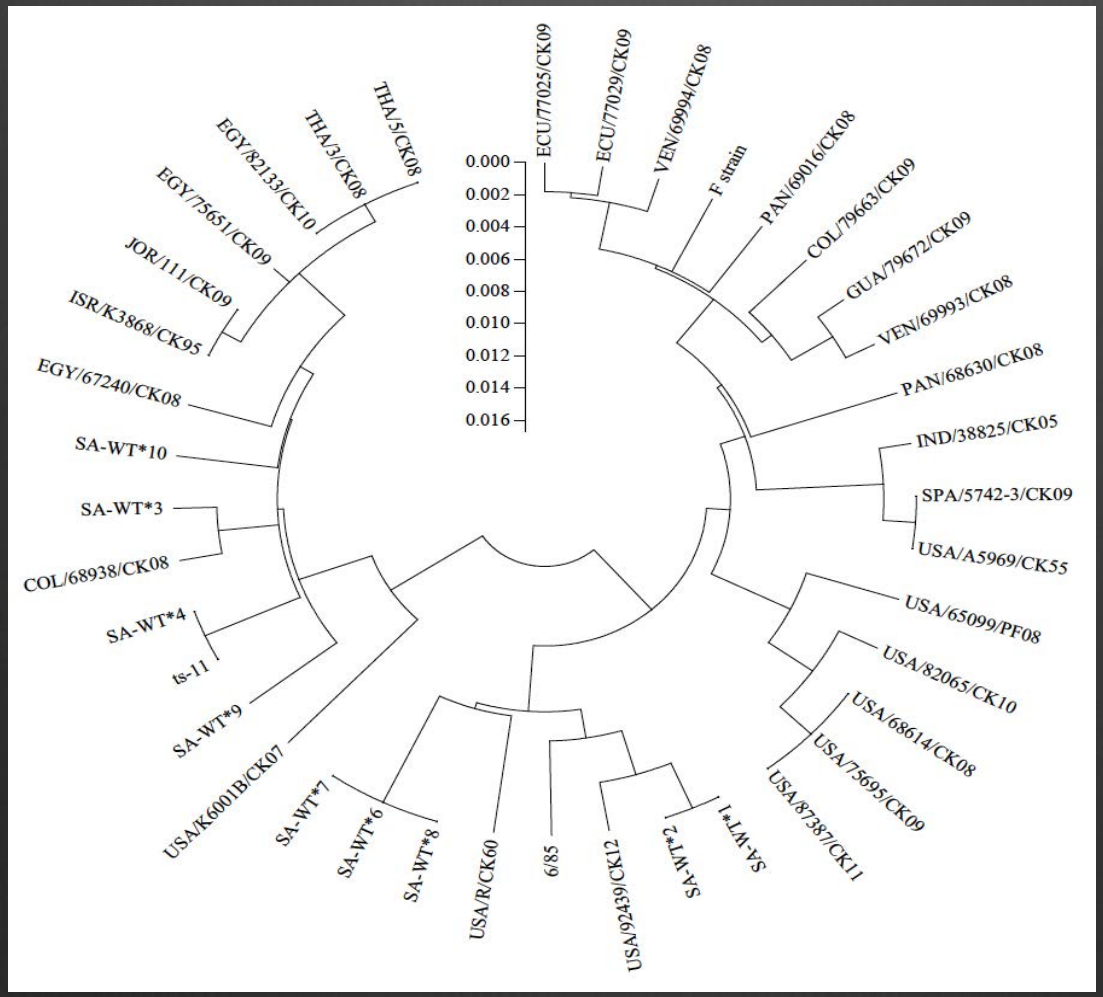
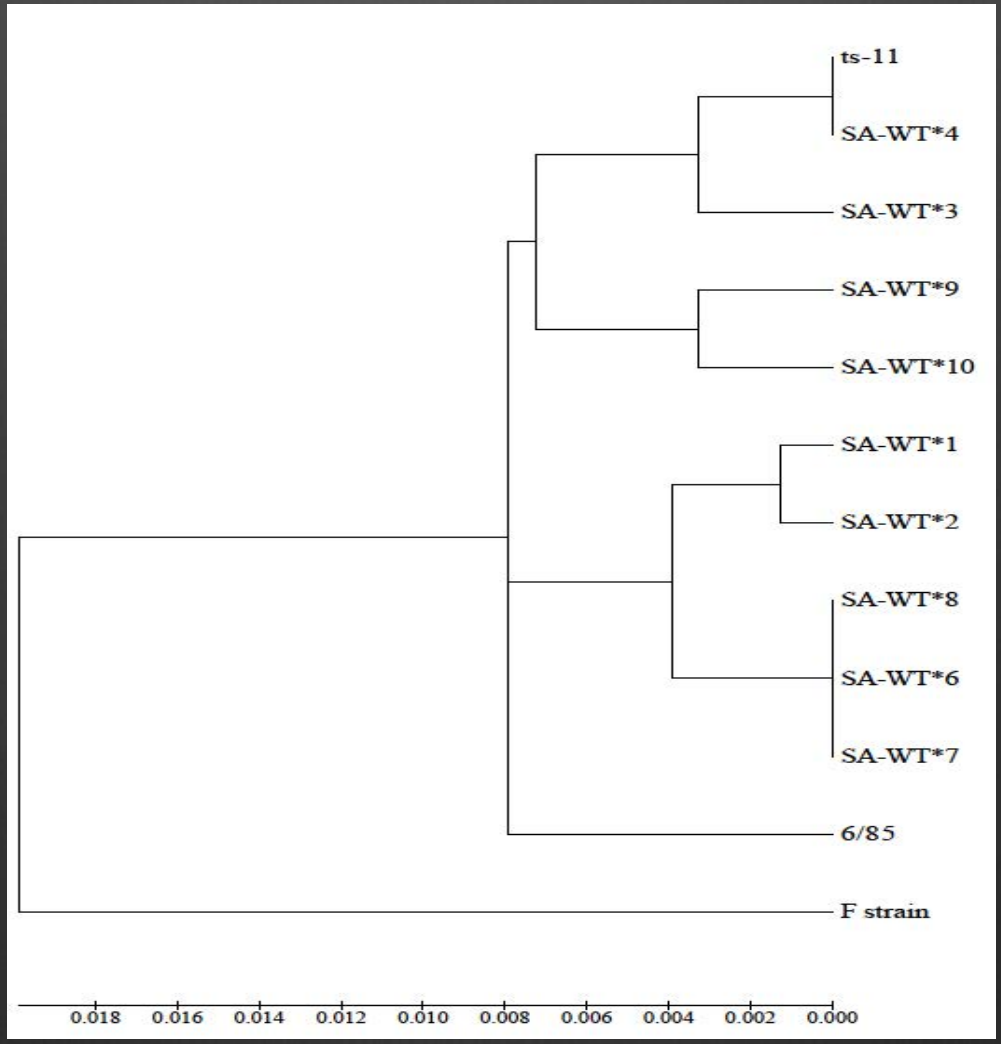
WT-Y/WT-10

Genotyping South African MG Isolates



Armour, N.K., et al (2013). *Avian Pathol*. doi:10.1080/03079457.2013.819486

Genotyping South African MG Isolates



Armour, N.K., et al (2013). *Avian Pathol*. doi:10.1080/03079457.2013.819486

MG epidemiology in Jordan

- Relatively large and developing chicken industry
- No national plan to control MG infection
- Farmers depend on vaccination and/or chemotherapy
- 70 % of broiler flocks that suffered from respiratory disease were positive for MG by ELISA

MG epidemiology in Jordan

- 2004-2005 vs 2007-2008 (n=24)
- None of the flocks were vaccinated

Gharaibeh, S., et al.,Avian Dis 55:212-216. 2011.

MG epidemiology in Jordan

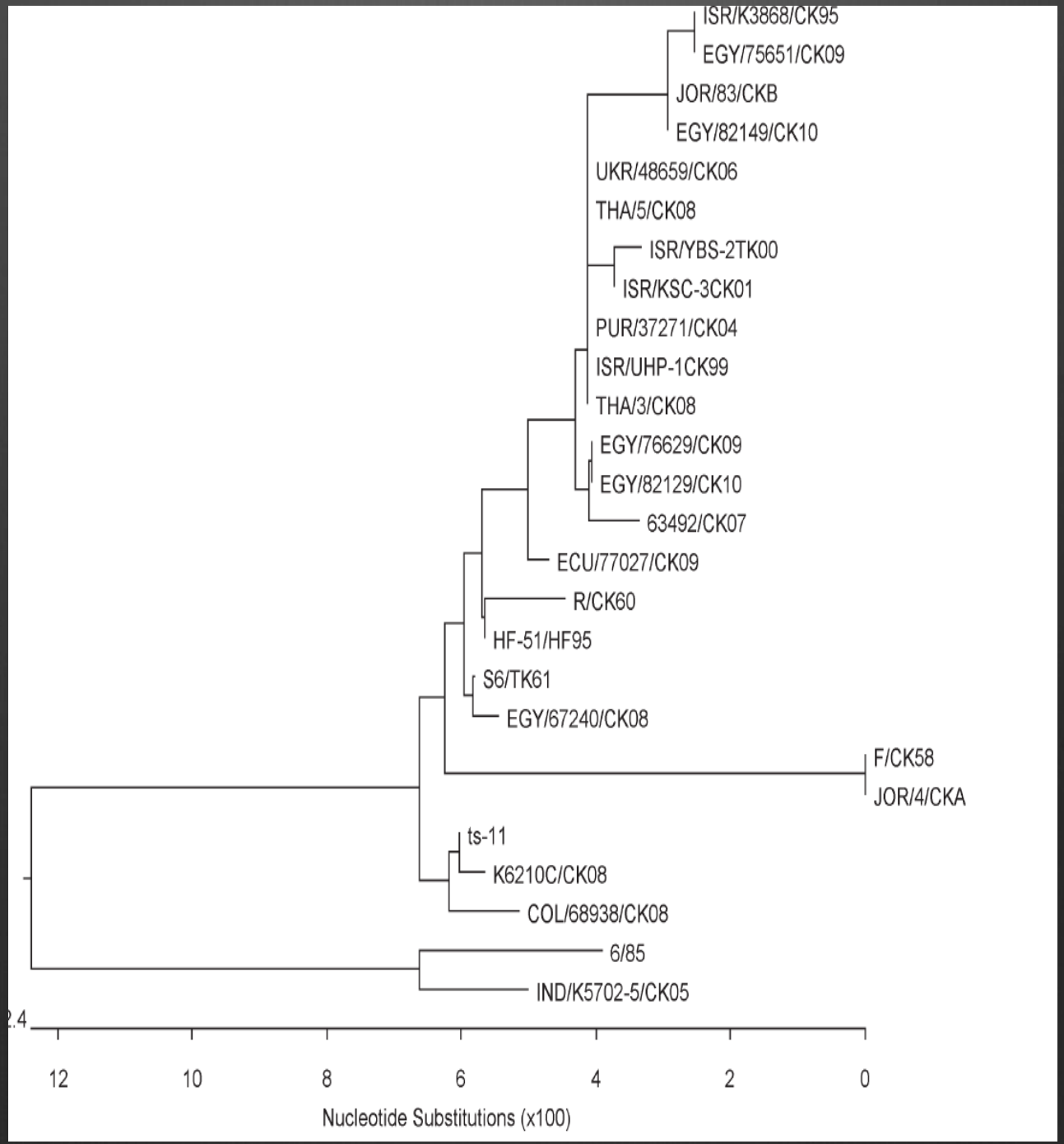
- 2004-2005 vs 2007-2008 (n=24)
- None of the flocks were vaccinated
- 21 isolates indistinguishable from F-strain

Gharaibeh, S., et al.,Avian Dis 55:212-216. 2011.

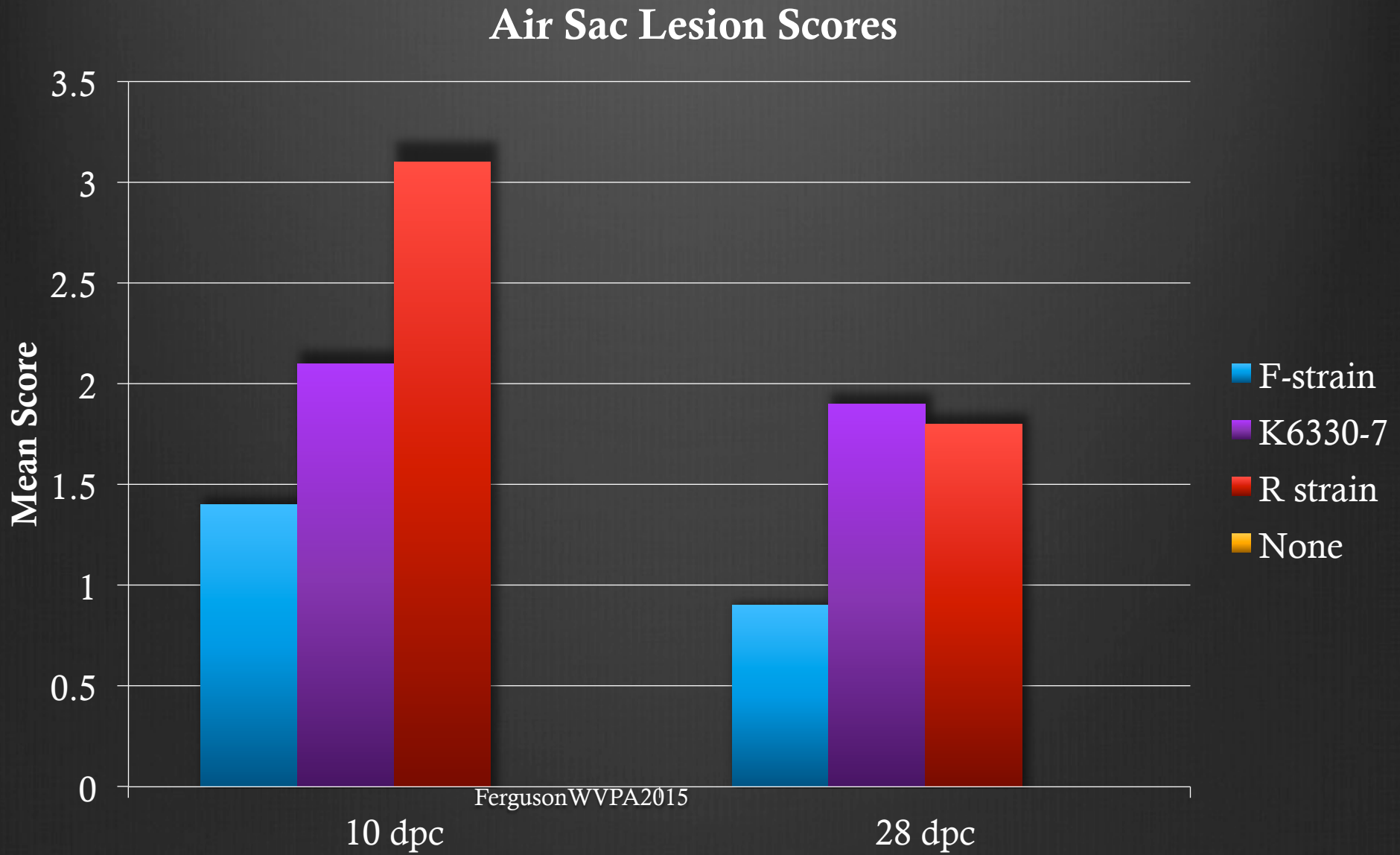
MG epidemiology in Jordan

- 2004-2005 vs 2007-2008 (n=24)
- None of the flocks were vaccinated
- 21 isolates indistinguishable from F-strain
- The most common vaccine used in Jordan in the early 2000's was F-strain
- In later years, F-strain availability was very limited

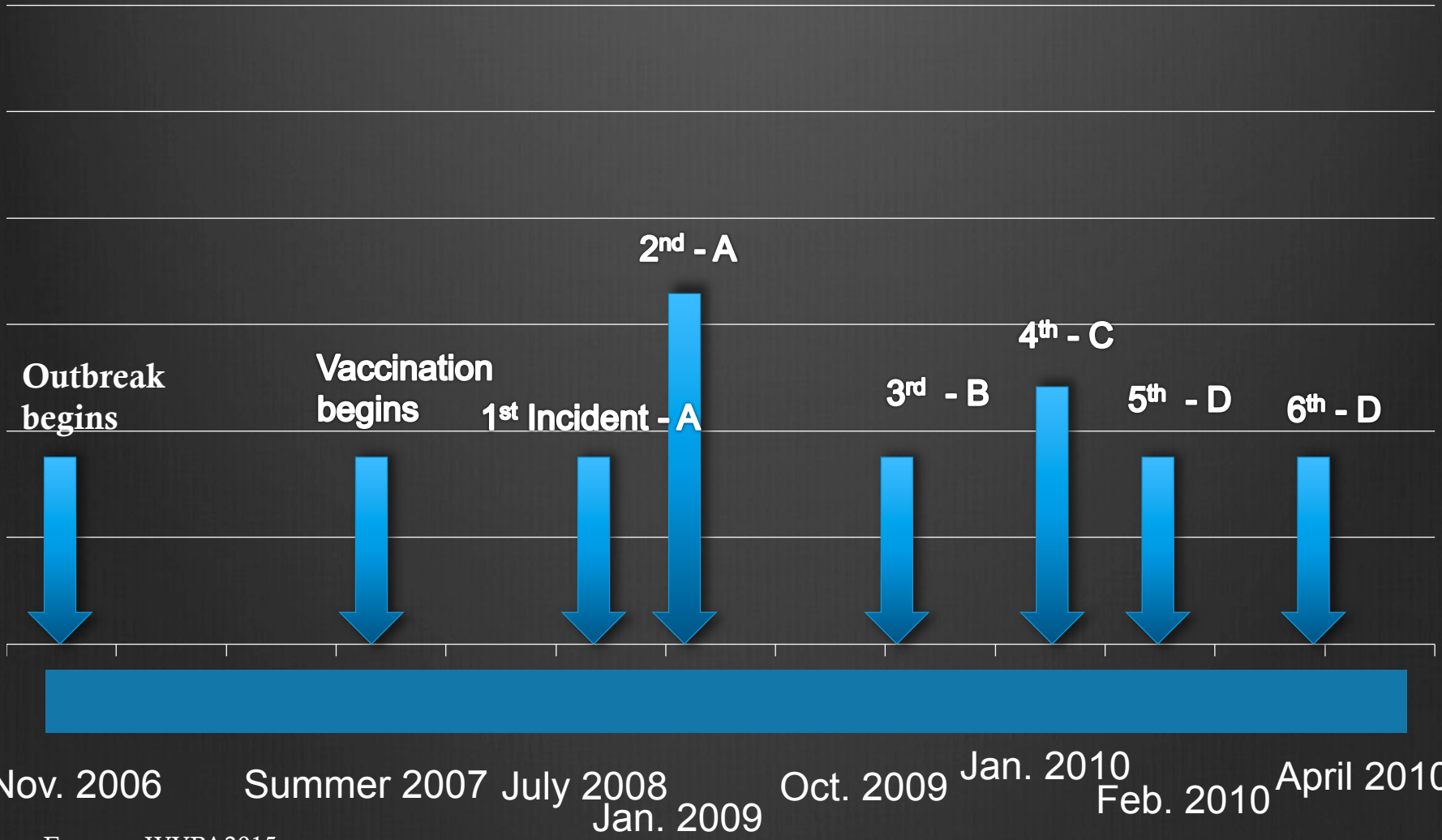
Gharaibeh, S., et al.,Avian Dis 55:212-216. 2011.


ISR/K3868/CK95
EGY/75651/CK09
JOR/83/CKB
EGY/82149/CK10
UKR/48659/CK06
THA/5/CK08
ISR/YBS-2TK00
ISR/KSC-3CK01
PUR/37271/CK04
ISR/UHP-1CK99
THA/3/CK08
EGY/76629/CK09
EGY/82129/CK10
63492/CK07
ECU/77027/CK09
R/CK60
HF-51/HF95
S6/TK61
EGY/67240/CK08
F/CK58
JOR/4/CKA
ts-11
K6210C/CK08
COL/68938/CK08
6/85
IND/K5702-5/CK05
12
10
8
6
4
2
0
Nucleotide Substitutions (x100)

Jordan F-Strain – Increased Virulence in Broilers



MG Outbreak in NE GA and ts-11 Vaccination

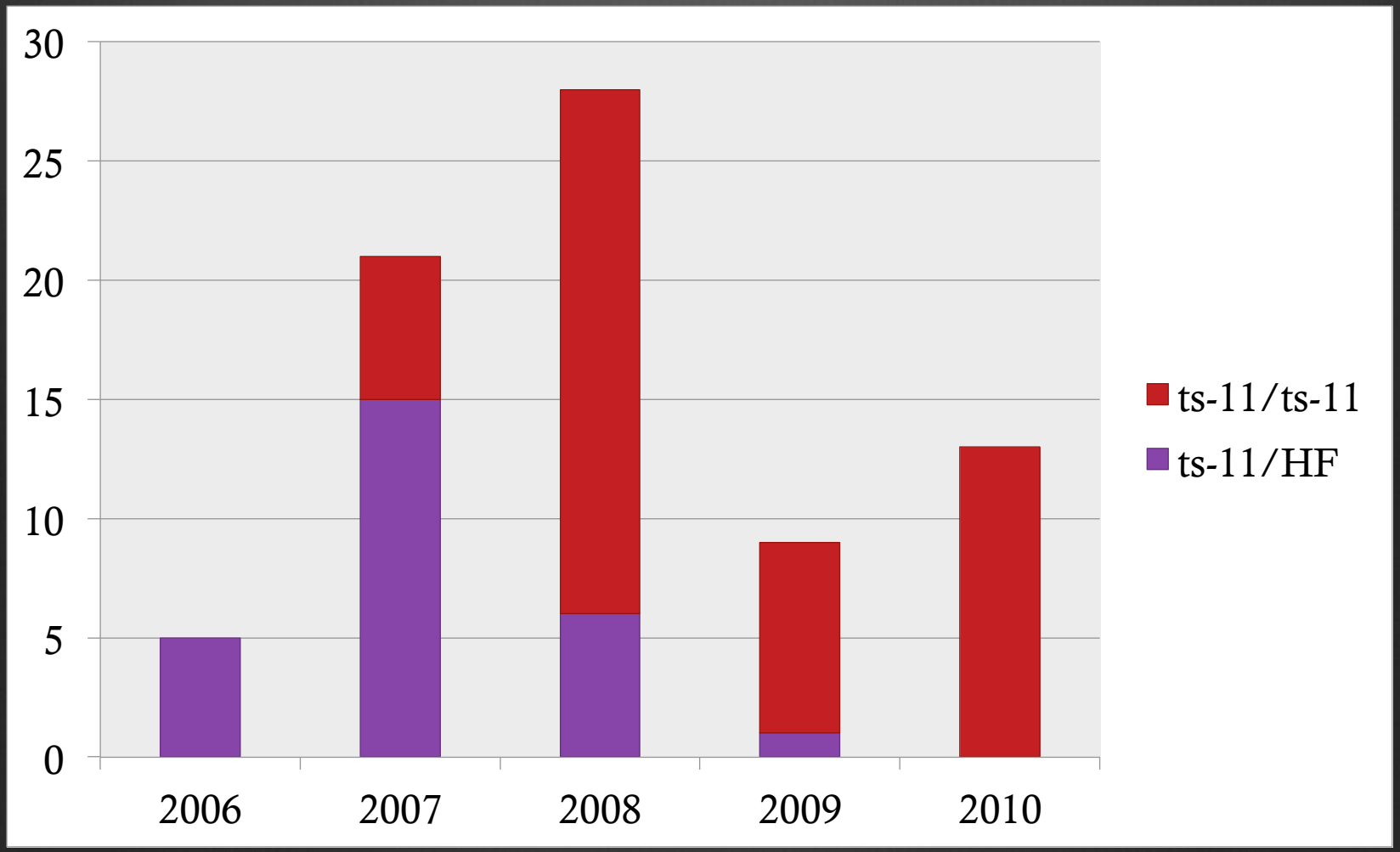


Increased Virulence and Vertical Transimssion

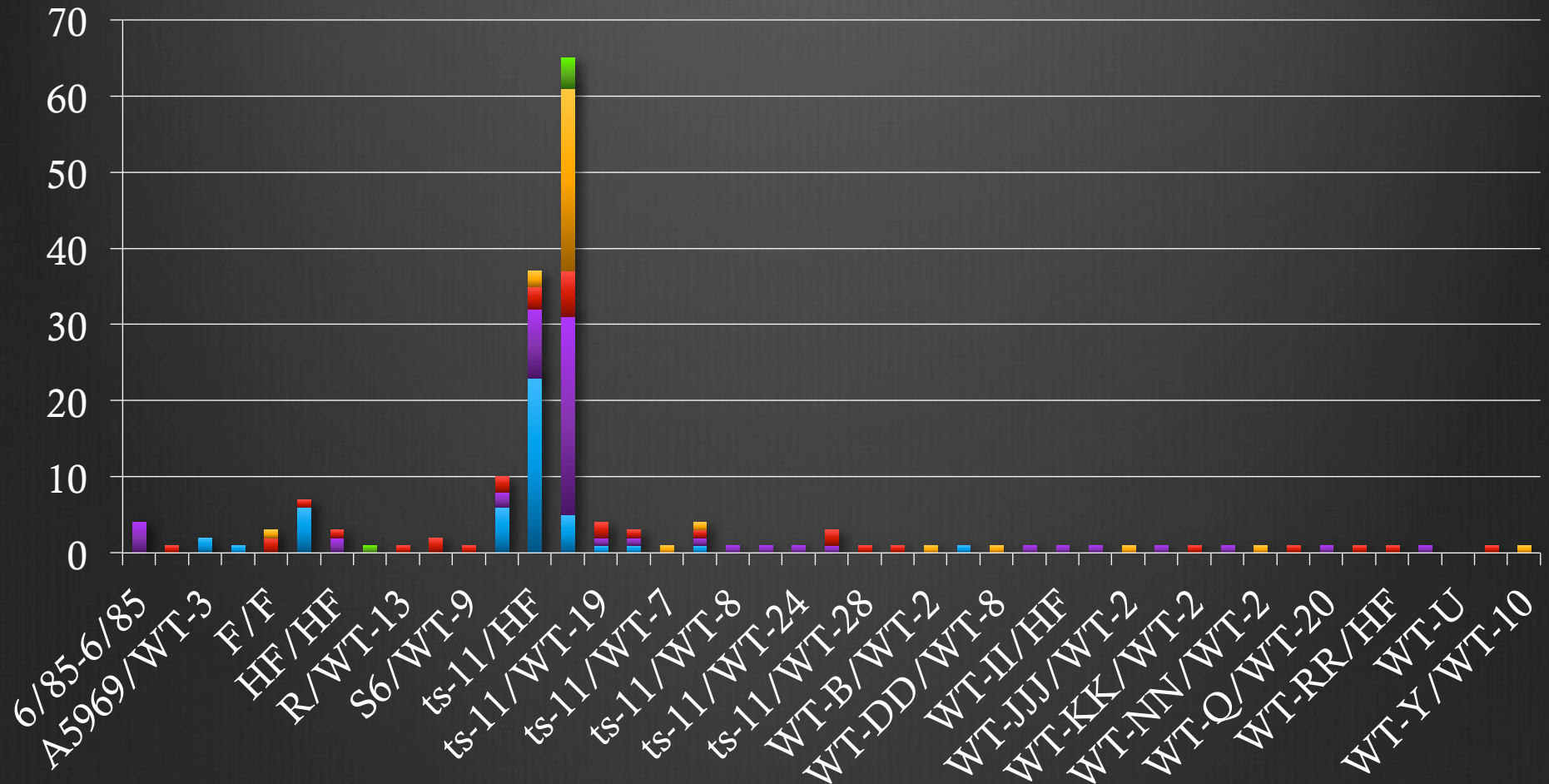
- Pathogenicity Trial
  - El Gazzar, M., Laibinis, V. & Ferguson-Noel, N. (2011). Characterization of a ts-11-like *Mycoplasma gallisepticum* Isolate From Commercial Broiler Chickens. *Avian Dis,* 55, 569-574.
- Vertical Transmission Trial
  - Armour, N.K. & Ferguson-Noel, N. (2015). Evaluation of the egg transmission and pathogenicity of *Mycoplasma gallisepticum* isolates genotyped as ts-11. *Avian Pathol*, 1-24. doi: 10.1080/03079457.2015.1044890

- http://www.gapoultrylab.org/wp-content/uploads/2012/05/Experience-in-Use-of-MG.pdf

MG – GA Broiler-type



MG Types


70
60
50
40
30
20
10
0
6/85-6/85
A5969/WT-3
F/F
HF/HF
R/WT-13
S6/WT-9
ts-11/HF
ts-11/WT-19
ts-11/WT-7
ts-11/WT-8
ts-11/WT-24
ts-11/WT-28
WT-B/WT-2
WT-DD/WT-8
WT-II/HF
WT-JJJ/WT-2
WT-KK/WT-2
WT-NN/WT-2
WT-Q/WT-20
WT-RR/HF
WT-U
WT-Y/WT-10

The Perfect Vaccine

- Safe
  - Reactivity
  - Transmission – horizontal and vertical
  - Reversion to virulence
- Efficacious
  - Duration of immunity
- Low Cost
- Easy to Administer and Transport
- Stable
  - On the shelf and in the birds

Characteristics of Mycoplasmas

- Colonize mucosal surfaces
  - Respiratory and urogenital tract
- Some can invade host cells

Mycoplasma pneumoniae On the surface of a cell
http://www.thenakedscientists.com/forum/index.php?topic=11352.0

Mycoplasma Pathogenesis

- Usually do not invade
- Attach to mucosal surfaces
- Emit toxic products and invoke host response
- Carrier state is usual

Mycoplasma pneumoniae On the surface of a cell
http://www.thenakedscientists.com/forum/index.php?topic=11352.0

Vaccines

- Natural exposure
- Naturally occurring avirulent strains
- *In vitro* attenuated strains
  - Serial passage
  - Chemical mutagenesis
  - Targeted mutagenesis
    - Gene deletion
  - Synthesis

Possible Factors in Pathogenesis of Mycoplasmas

- Attachment
- Ciliostasis
- Depletion of cell nutrients
- Local toxins
- Penetration of cells?
- Stimulation of immunopathological reaction
- Effects on lymphoid cells & macrophages
- Antigen variation – immune evasion

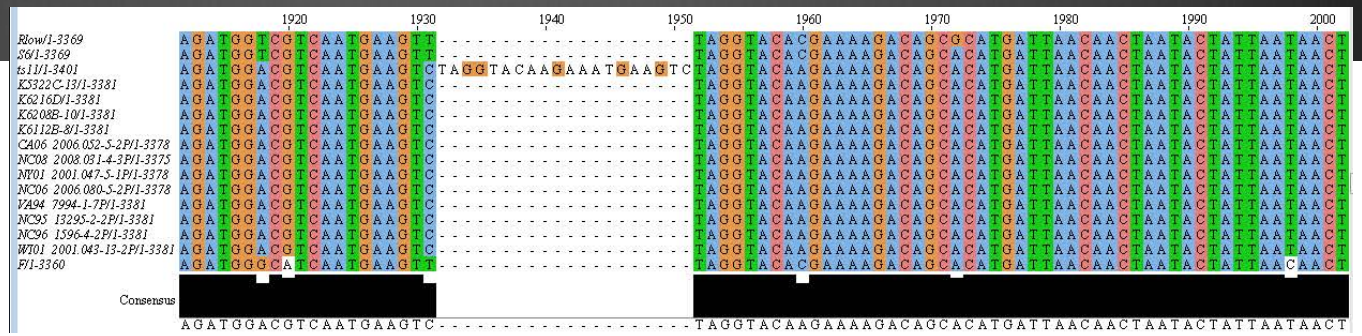
Vaccines

- Inactivated oil-emulsion bacterins
- Recombinant MG Vaccine
- Live vaccines
  - F Strain
  - ts-11
  - 6/85

ts-11

- Temperature sensitive mutant
- Developed in Australia
- Product is frozen
- Administer by eye drop

gapA


1920
1930
1940
1950
1960
1970
1980
1990
2000
Rlow/1-3369
S6/1-3369
ts11/1-3401
K5322C-13/1-3381
K6216D/1-3381
K6208B-10/1-3381
K6112B-8/1-3381
CA06_2006.052-5-2P/1-3378
NC08_2008.031-4-3P/1-3375
NY01_2001.047-5-1P/1-3378
NC06_2006.080-5-2P/1-3378
VA94_7994-1-7P/1-3381
NC95_13295-2-2P/1-3381
NC96_1596-4-2P/1-3381
WI01_2001.043-13-2P/1-3381
F/1-3360
Consensus

ts11
K5322C-13
K6216D
0.14
K6208B-10
K6112B-8
0.08
CA06_2006.052-5-2P
0.96
NY01_2001.047-5-1P
VA94_7994-1-7P
0.00
NC95_13295-2-2P
0.00
NC96_1596-4-2P
0.21
0.00
WI01_2001.043-13-2P
0.03
NC06_2006.080-5-2P
0.16
NC08_2008.031-4-3P
0.57
Rlow
0.79
0.80
S6
0.96
4.93
F

The Perfect Vaccine

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  - Reactivity
  - Transmission – horizontal and vertical
  - Reversion to virulence
- Efficacious
  - Duration of immunity
- Low Cost
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- Stable
  - On the shelf and in the birds

AVIAN DISEASES 48:91–99, 2004

Safety and Efficacy of the Avirulent Mycoplasma gallisepticum Strain K5054 as a Live Vaccine in Poultry

N. M. Ferguson, V. A. Leiting, and S. H. Kleven[A]

Department of Avian Medicine, College of Veterinary Medicine,
University of Georgia, Athens, GA 30602–4875

Received 23 May 2003

Background

- K5054 is a naturally occurring immunogenic isolate with low virulence
- ‘Atypical’ MG outbreak in turkey breeders in late 2000

K5054

- K5054 similar to house finch isolates
  - RAPD
  - Sequence analysis of selected genes
    - *pvpA*
    - lipoprotein gene (LP)
    - *mgc1 (gapA)*

GREENPEACE

Search

To: Office of the Registrar : Genetically Modified Organisms Act, Private Bag X973, Pretoria 0001, Fax: 012 319 6329

Say NO to Release of GMO Vaccine in South Africa

Campaign created by
Delwyn Pillay

Thank you

Naola Ferguson-Noel, DVM, MAM, PhD

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