



Animal &
Plant Health
Agency

BVPA gamebird respiratory project – final report

David Welchman, APHA Avian Expert Group

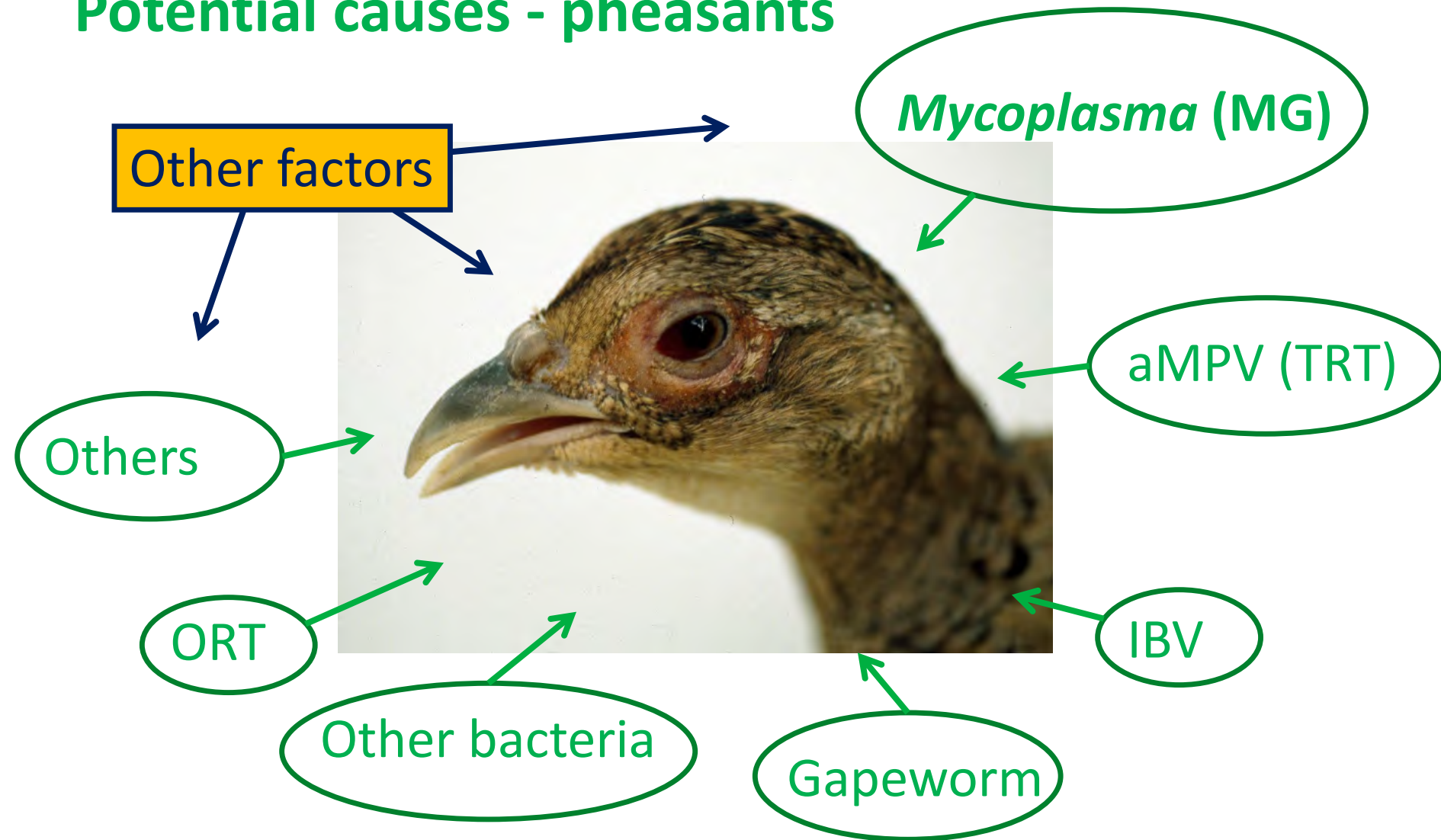
John Tasker, Janet Bradbury and members
of BVPA working group

BVPA Spring meeting 15 March 2019

Gamebird respiratory disease - project

- Background – apparent worsening situation with ‘Mycoplasma’ in gamebirds
- Cases unresponsive to treatment
- Request for coordinated approach
- **2016 – BVPA gamebird working group set up**

Potential causes - pheasants



BVPA working group plan 2016-18

- **Evidence base** for current situation
- **Investigate field cases**
 - Standard protocol
 - Differential diagnosis
 - Detailed *M. gallisepticum* investigation
 - MICs - Three standard antimicrobials + two extra in 2018 – standard method
 - Strain typing

Results of testing

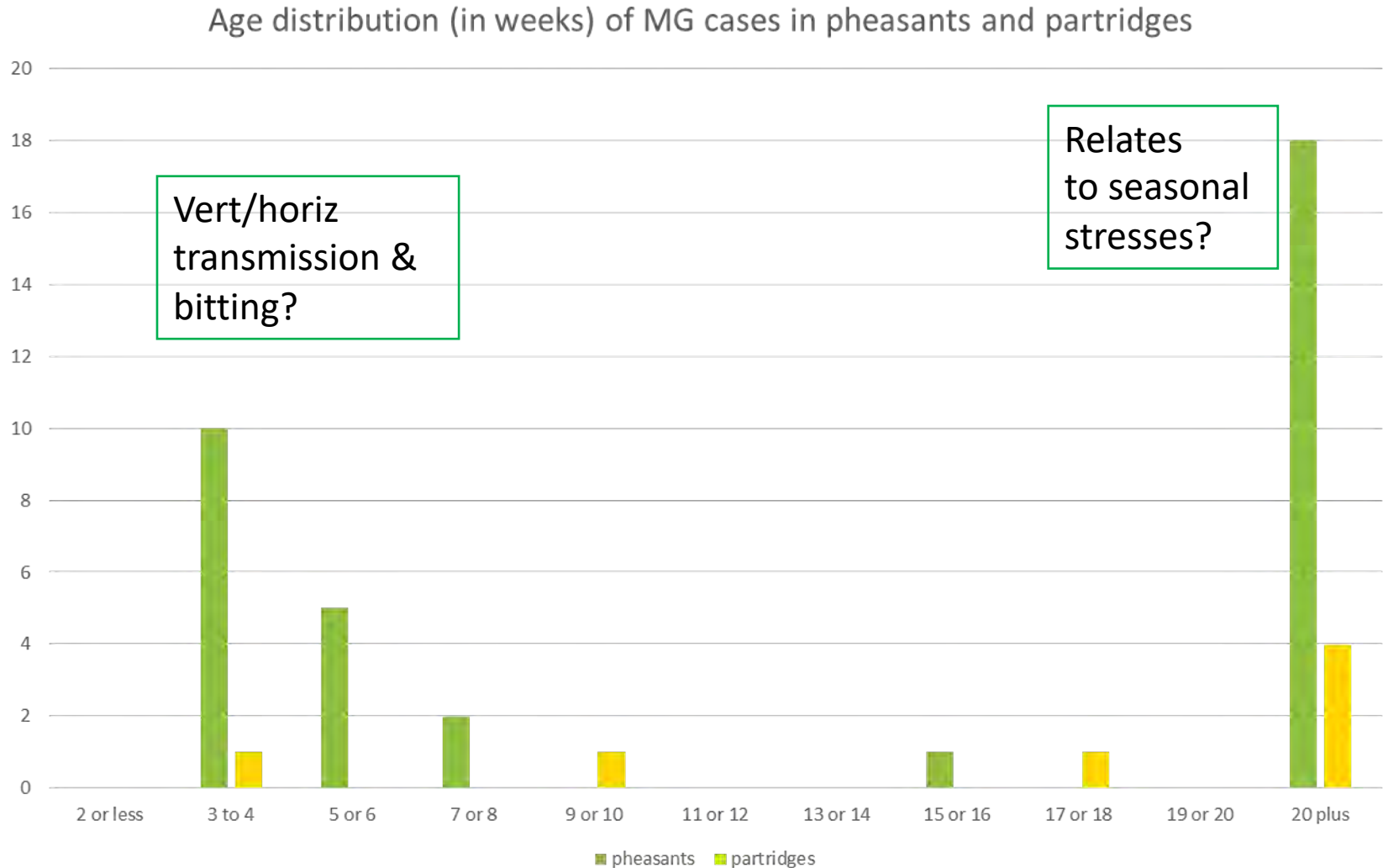
Summary of case numbers 2016-18

	2016	2017	2018
Number of cases	17	29	24 (total = 70)
Number of partridge cases	3	5 + one grey	5 (total = 14)
Number of cases where MG isolated & MIC done (+PCR pos)	15	25 (+ two MG PCR pos)	15 (total = 55) (+ six MG PCR pos) Total MG positive = 62
Number of differentials done	5	25	21 (total = 51)
Age range		20 days to adult	3 weeks to adult

Younger birds being affected



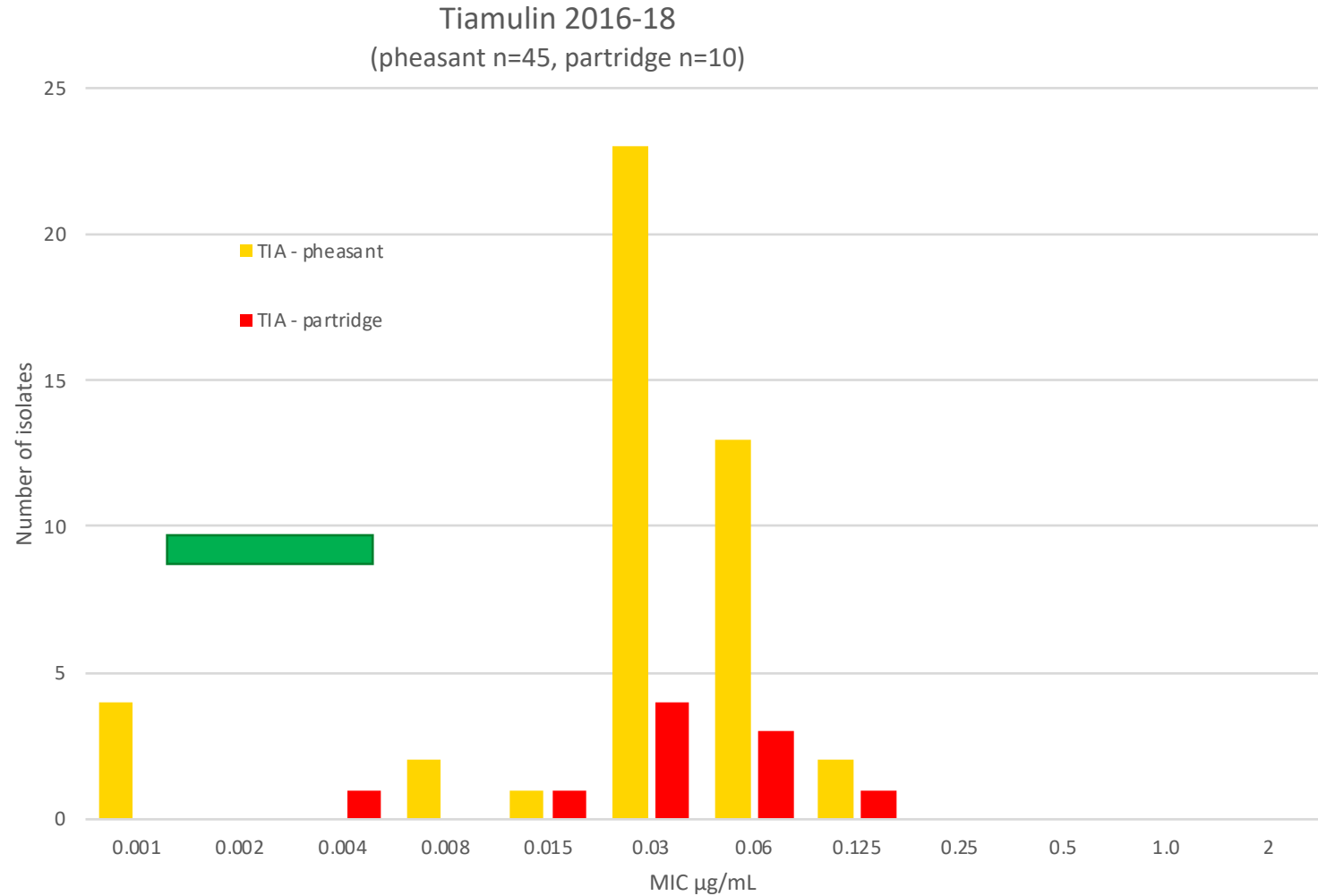
MG age distribution (weeks) – MIC done



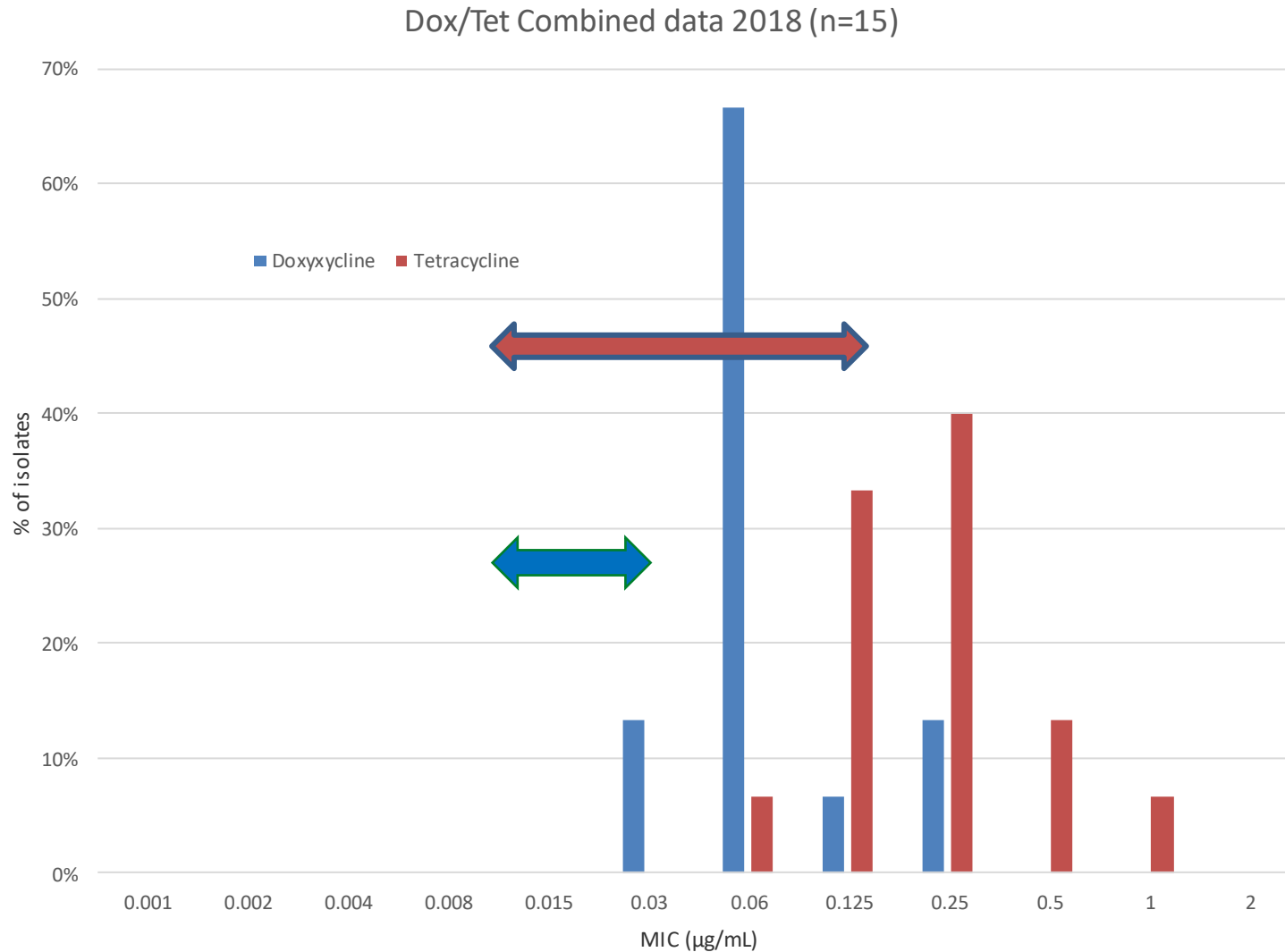
MIC results – tiamulin, tylosin, tylvalosin (thanks to John Tasker for analysis)

2016-18 combined pheasant + partridge						
Antibiotic	n	Geomean	range	MIC90	mode	Ref MIC
TIA	55	0.03	<0.002 - 0.125	0.06	0.03	0.001 - 0.004
TYL	55	1.06	0.008 - >1.0	2	2	0.002 – 0.015
TVN	55	0.25	0.008 - 1.0	0.5	0.5	0.002 – 0.015

Tiamulin MICs pheasant & partridge

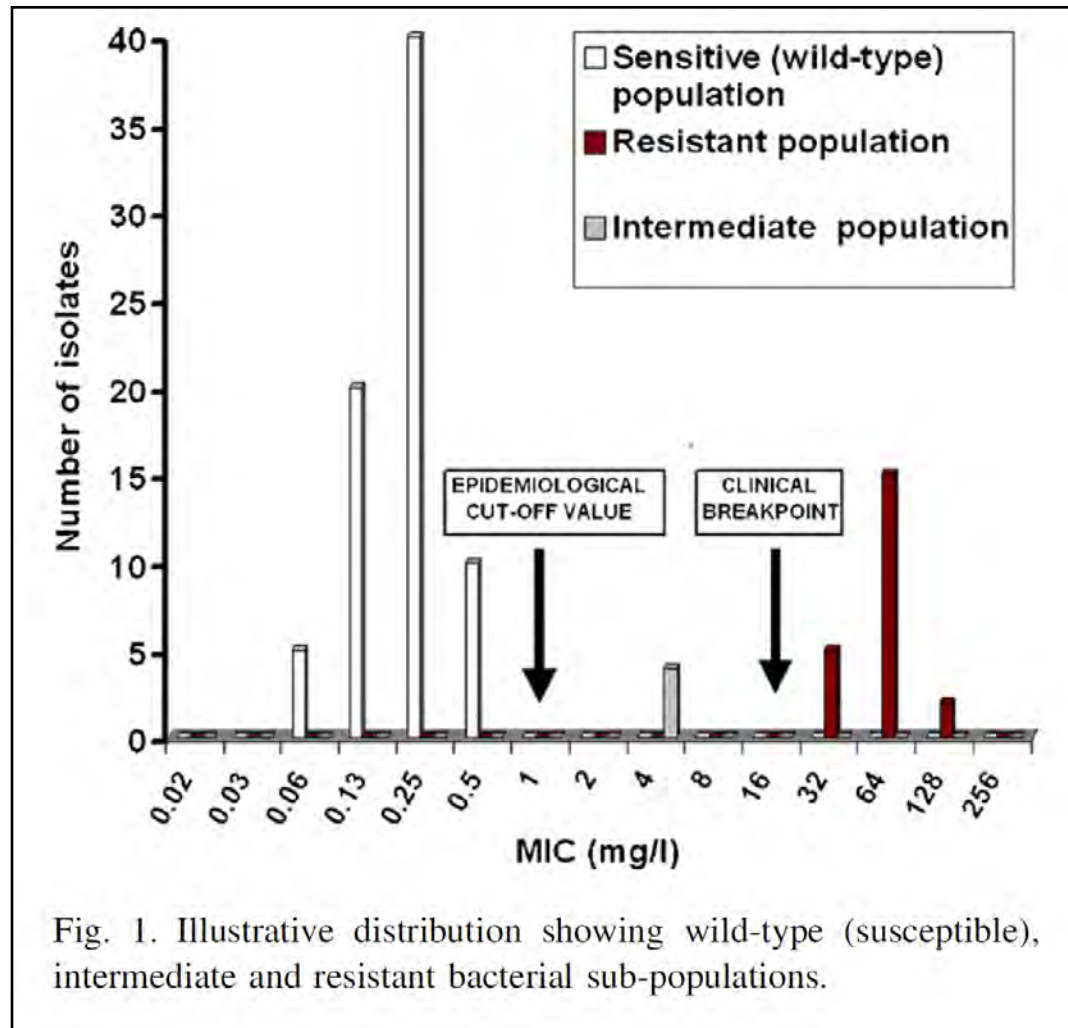


Doxy and Tet MICs 2018



MIC breakpoint illustration

(Bywater et al 2006, n=102)



MICs relating to clinical response (2016-18)

	Responsive cases	Non-responsive cases
Tiamulin (n = 22)	0.002 – 0.06	0.002 – 0.06
Tylosin (n = 0)	-	-
Tylvalosin (n = 12)	0.25 – 0.5	0.015 – 1.0
Doxycycline (n = 1)	-	0.06
Tetracycline (n = 0)	-	-

- Route of admin (water/feed) may have an effect
- Apparent overlap in MICs between responsive and non-responsive cases

MICs and other pathogens in non-responsive cases (2016-18) – nb max 5 birds per case

Case ref	Treatment & MIC	Other pathogens detected
16L - 2104	Tiamulin 0.002	<i>Avibacterium avium</i>
16L - 2135	Tiamulin 0.002, Tylvalosin 0.015	IBV, ORT, <i>Avibacterium avium</i>
17L - 4248	Tiamulin 0.06, Tylvalosin 1.0	<i>E. coli</i>
17L - 4568	Tiamulin 0.03, Tylvalosin 0.5	<i>E. coli</i>
17L - 5668	Tiamulin 0.06	<i>E. coli</i>
417	Tylvalosin 0.25	ORT
684	Tiamulin 0.04	<i>E. coli</i>
1792	Tylvalosin 0.25	ORT, <i>Aeromonas hydrophila</i>
17L - 3035	Tiamulin 0.03, Tylvalosin 0.25	<i>E. coli</i> mixed*
17L - 3927	Tiamulin 0.125	(coliforms)*

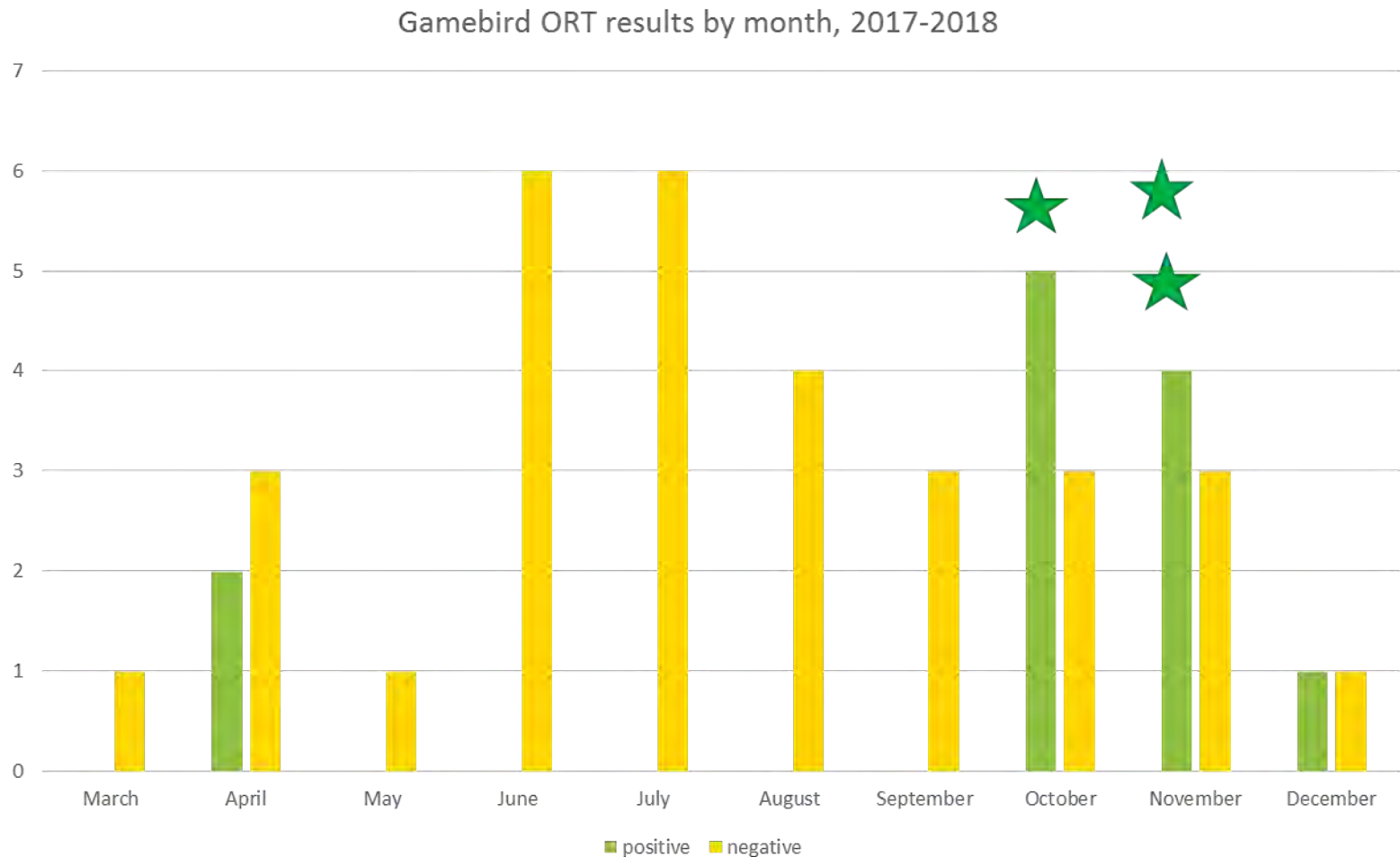
Yellow highlight = MIC 90 or greater

MICs and other pathogens in non-responsive cases (2)

Case ref	Treatment & MIC	Other pathogens detected
17L - 3788	Tiamulin 0.06	(coliforms)*
17L - 4327	Tiamulin 0.03	Not tested
17L - 4007	Tiamulin 0.06	(coliforms)*
4888 (2018)	Tiamulin 0.03	<i>E. coli</i>
4921	Tiamulin 0.06, Tylvalosin 0.5	<i>E. coli</i> , <i>A. gallinarum</i>
5464	Tiamulin 0.06	<i>E. coli</i> , <i>K. pneumoniae</i>
6052	Tylvalosin 0.5	Mixed flora
7947	Tylvalosin 0.015, Doxycycline 0.06	ORT, IBV, <i>E. coli</i>

*bacteriology only, no PCRs

ORT (PCR/culture +ve) results 2017-2018 (*partridge)



MICs relating to source of birds (2017-18)

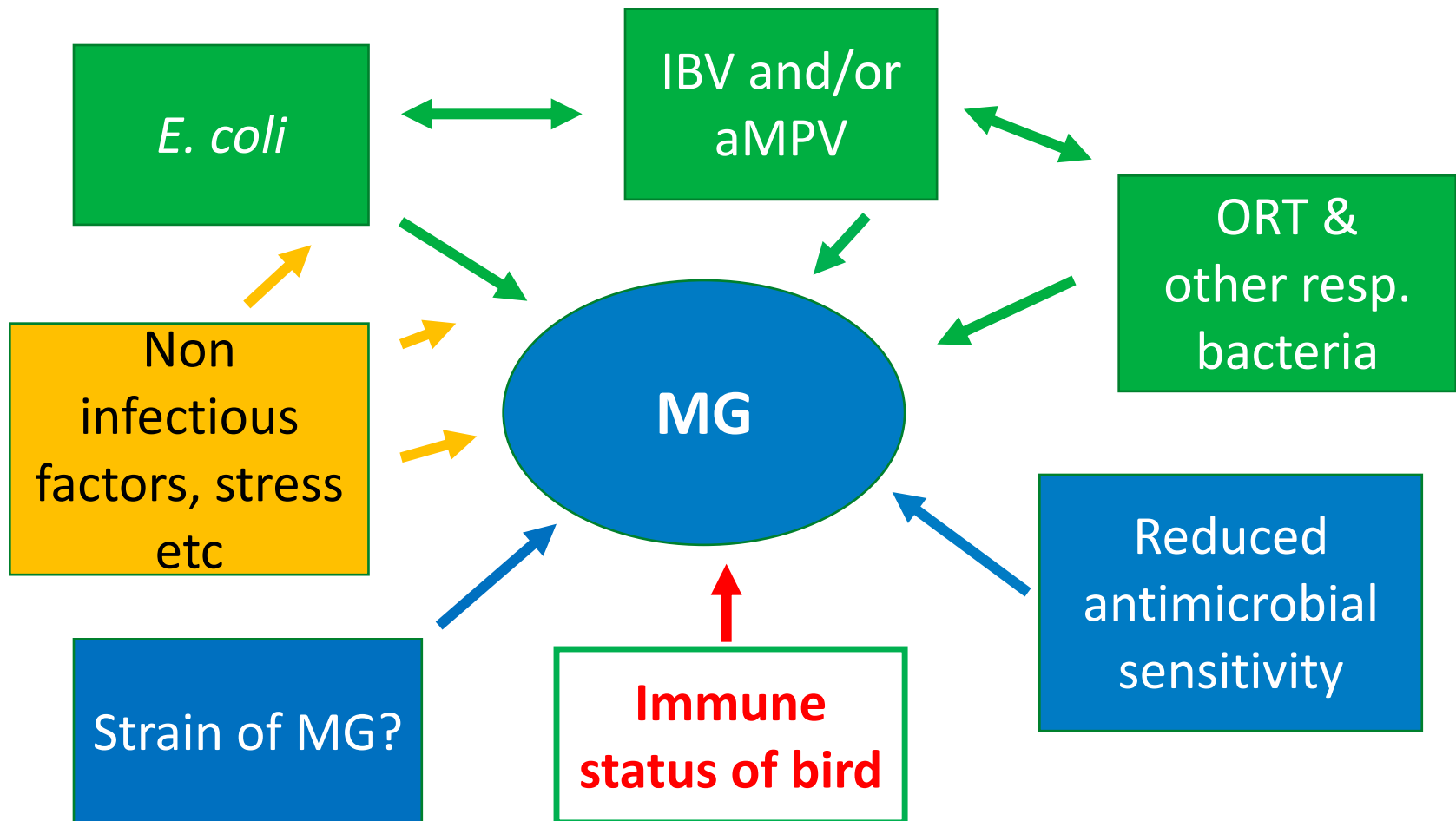
	Homebred/ Overwintered (n = 6)	Caught up (n = 4)	UK bought (n = 11)	France (n = 3)
TIA	0.002 – 0.125	0.03-0.06	0.004 – 0.06	0.03 – 0.06
TYL	0.06 - >1.0	0.5 – >1.0	0.008 - >1.0	1.0 - >1.0
TVN	0.015 – 0.5	0.125 – 0.5	0.015 – 1.0	0.25

- Source refers to this year's birds, not previous years'
- Possible resistant ?clones or ??strains - widespread

Conclusions so far

- MG is a consistent finding throughout
- There are some MG isolates that are less responsive to antibiotics
- Clinical response doesn't necessarily relate to MIC
- Other potential pathogens may play a role – synergism with MG (respiratory complex)
 - E. coli
 - ORT
 - IBV, aMPV
 - Other bacteria
- Role of non-infectious factors – stress, weather etc – in the mix

What is the explanation of non-responsive (intransigent) respiratory disease?

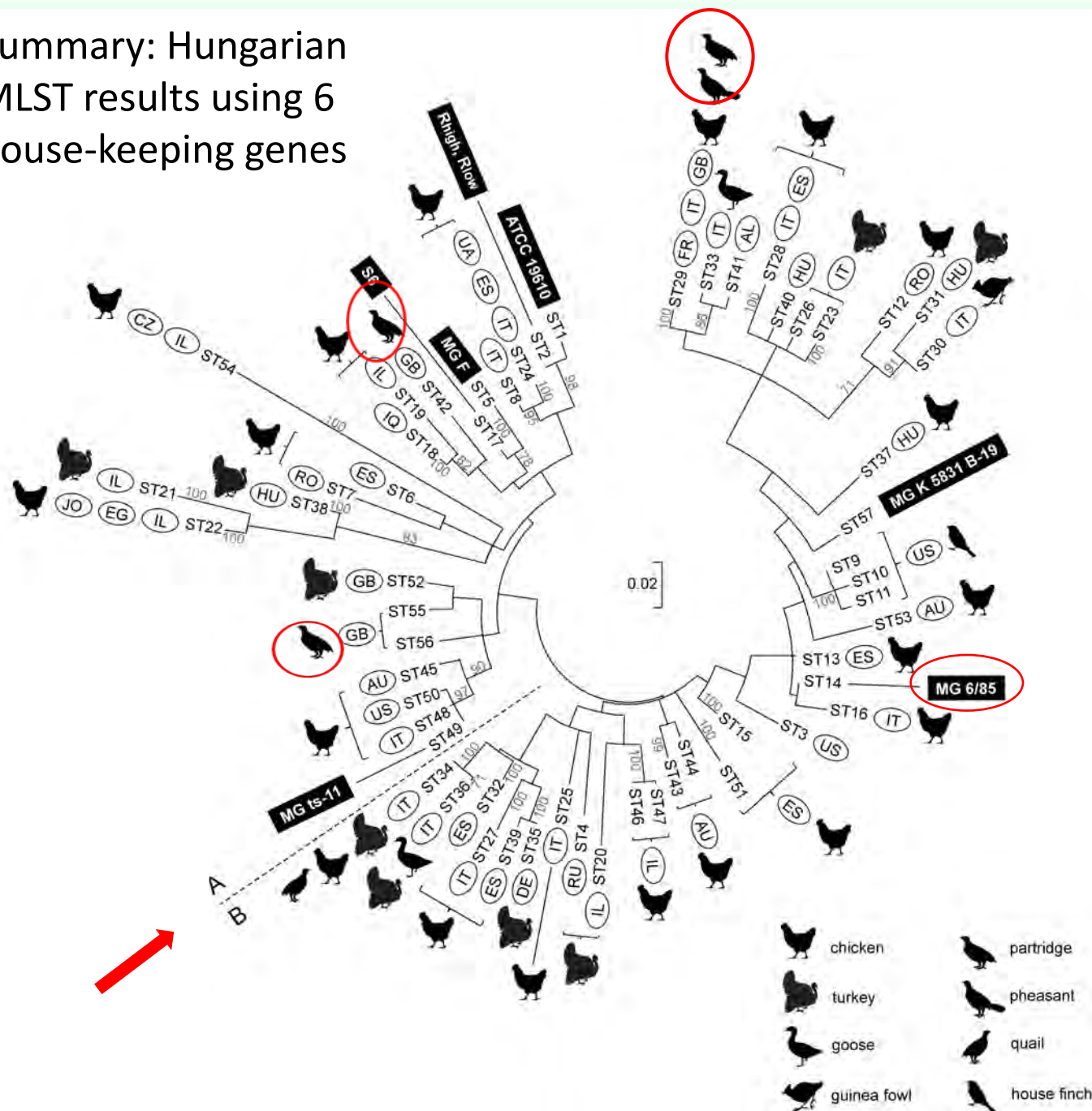


Strain typing – use of Multilocus sequence typing (MLST) – Janet Bradbury

- Allows ‘identification of clones within populations of pathogenic microorganisms’
 - Database can be interrogated electronically - powerful resource for epidemiology
 - Unambiguous procedure to characterise isolates of bacterial species using sequences of (usually) 7 house-keeping genes
 - Widely used for other bacteria e.g. *Campylobacter*, *Neisseria*
-

Results from Hungary

Summary: Hungarian MLST results using 6 house-keeping genes



Conclusions from Hungarian MLST

Miklós' comment:

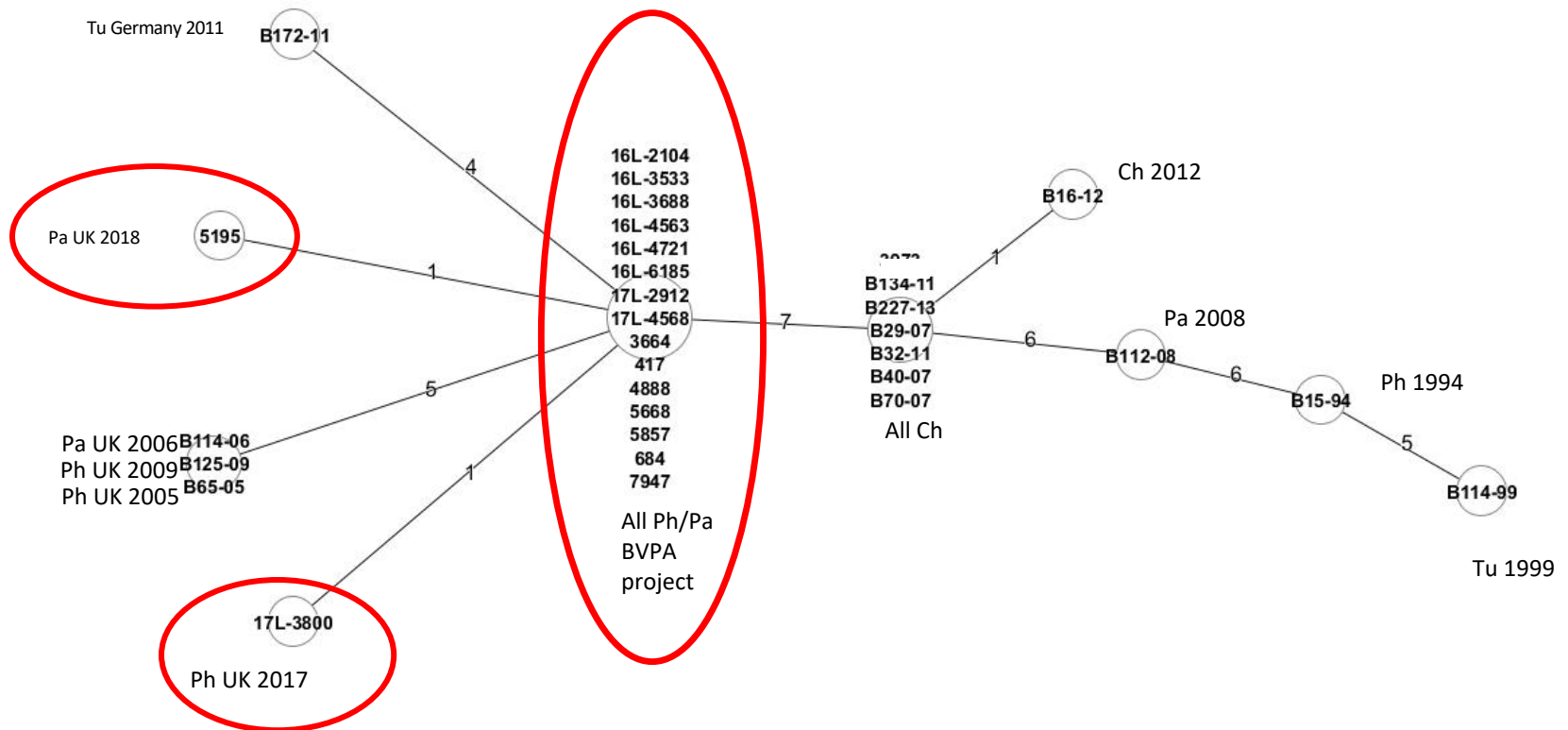
“It seems that one (dominant) genotype/strain is circulating in your gamebird population”



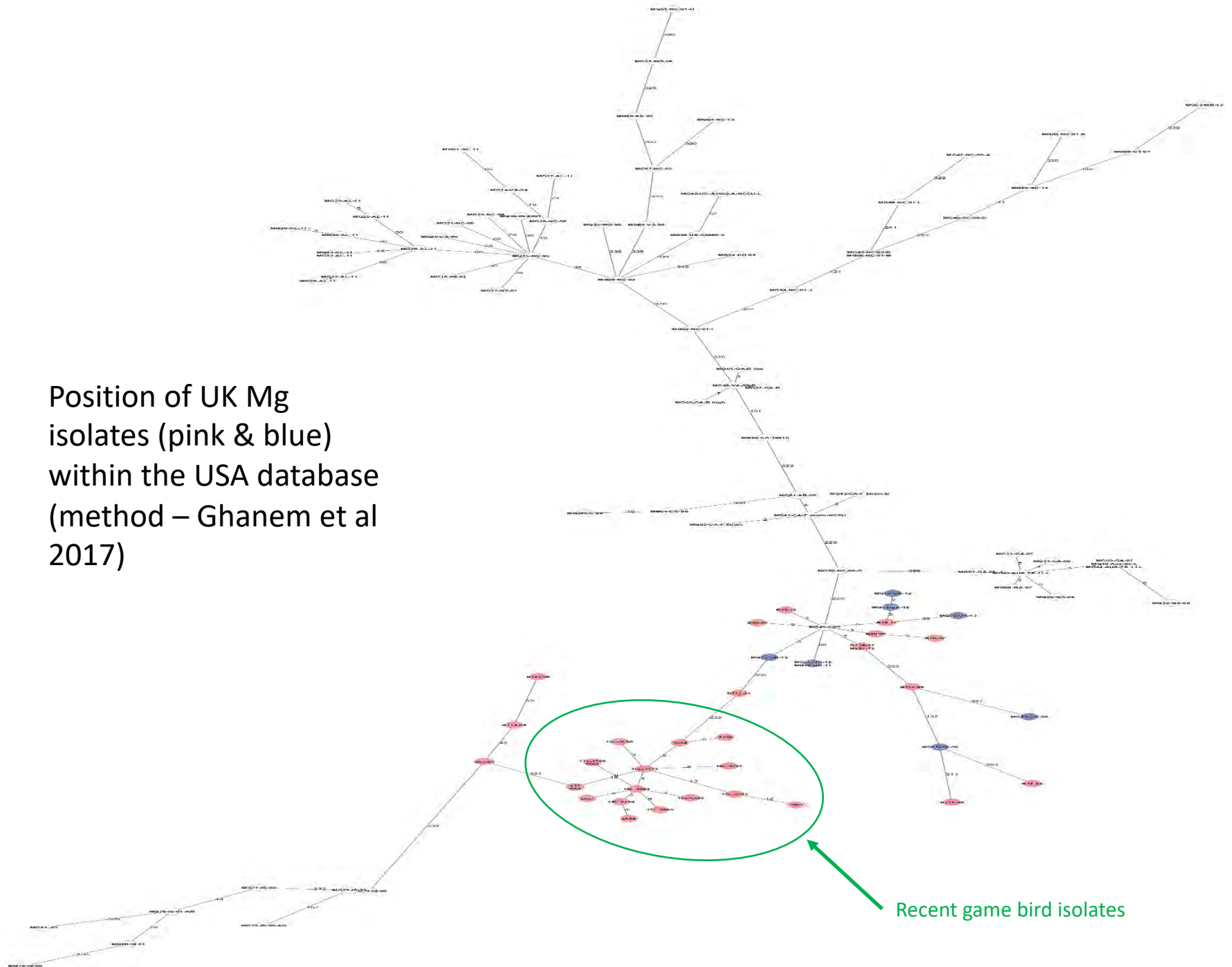
Further analysis of samples in USA

- Whole genomes sequenced, assessed for quality, trimmed, assembled with reference to reference strain Mg R_{low}
 - 7 housekeeping genes analysed in conventional MLST
 - (different gene set to that used in Hungary)
 - 425 genes analysed in cg MLST - 300 base sequences from each
 - 19 project strains analysed (13 pheasant, 6 partridge)
 - Results still being assessed
-

Results based on conventional 7 gene MLST



Position of UK Mg
isolates (pink & blue)
within the USA database
(method – Ghanem et al
2017)



Summary of MLST results

- So, in summary, the Hungarians used an MLST based on 6 housekeeping genes, the Americans used an MLST based on 7 housekeeping genes (different genes to those used by the Hungarians).
 - The results, together with those of the 425 core gene MLST show that the recent UK game bird isolates belong to a 'clonal cluster'. As to whether these are the same strain, it depends on how one defines a strain but they are all closely-related genotypically.
 - The recent UK game bird isolates were different to earlier game bird samples (2005 - 2011).
-

Where do we go from here?

- Evidence base from project
- BVPA working group recommendations
 - Good management & stockmanship
 - Investigation & diagnosis
 - Veterinary input
- Need more consideration for longer term control approach

BVPA working group recommendations for Mycoplasma management in gamebirds – December 2018

The BVPA gamebird Mycoplasma project has confirmed that *Mycoplasma gallisepticum* (MG) plays a central role in respiratory disease in pheasants and partridges. Its effects vary depending on the presence of other factors. These include the presence of other infectious agents (viruses, bacteria) affecting the respiratory tract, other intercurrent diseases that can stress the birds and also a range of non-infectious factors. Many of these non-infectious factors relate to the management of the birds, including stocking density, hygiene, feeding, origin of the birds, whether birds from different sources are mixed etc.

In the light of the above, the BVPA working group recommends the following:

Breeding stock

1. The source of the breeding stock should be known and breeding birds should preferably be over-wintered on the breeding site or in a safe location and transported directly to the breeding site.
2. Breeding birds, both cocks and hens, should be managed, handled and transported in a stress free manner leading up to and during the breeding season.
3. Any outbreak of respiratory disease in breeding stock (cocks or hens) should be investigated. Not all outbreaks involve MG and many outbreaks are multifactorial and may or may not involve MG. If MG is confirmed action should be taken to minimise transmission either horizontally to other stock or vertically through the egg. Any breeding birds displaying clinical signs of respiratory disease, including conjunctivitis, should be culled.

Thank you to -

- 
- MSD, GFA, NGO, BVPA Trust and vet practices for funding MG work and MICs
 - APHA for differential testing & support
 - Practices for submitting cases and contributing further information

Vaccination?

Vaccinated cases (where info provided)

Vaccine used	No. of cases
Poultvac MG-Bac (inactivated)	7
MG 6/85 (live)	2
Autogenous Mg	3 + 1 parent flock
aMPV/ART	3
IBV	2

- vaccination not always protective
 - against clinical signs
 - probably not protective against transmission
 - may be of value in some situations