

Recent experiences with differentiating *Pasteurella multocida* outbreak strains

BVPA Winter Meeting 2024

Dr Kenton Hazel



Nicholas

Idea

Develop



Research

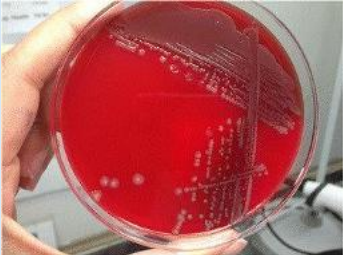
Exper

Creativity

Improvement

Concept





Fowl Cholera (avian pasteurellosis)



- Fowl cholera is a commonly occurring avian disease that in its peracute form is one of the **most virulent and infectious diseases** of poultry.
 - Caused by *Pasteurella multocida* which is readily identified by culture from affected birds – grows on blood agar but generally not on MacConkey agar.
 - Gram negative, non motile, non-spore forming rods. Grows in both aerobic and anaerobic conditions.
 - Fowl Cholera usually occurs in older birds with the first sign frequently being mortality – turkeys tend to be very severely affected. Typically presents as dead birds with blood in their mouths.
 - *Pasteurella multocida* infections occur in a wide range of species including humans but most mammalian isolates will not infect poultry and fortunately avian strains will generally not infect humans. Humans tend to get infected from animal bites.



Lung lesions



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Fowl Cholera - disease situation (turkey breeder perspective)

■ Europe

- Historically a few sporadic cases of Fowl Cholera.
- Generally well controlled through the use of killed autogenous vaccines – few commercial vaccines available in Europe any more – Landavax.
- Some companies don't vaccinate for Fowl Cholera

■ USA

- Historically extremely problematic in turkey companies
- Has required the use of multiple doses of vaccine (autogenous and commercial) including live vaccines.
- Currently being better controlled across much of the turkey breeder sector due to a major focus on rodent control and vaccination.



Example of US vaccination programme

8 Weeks: Autogenous vaccine

9 Weeks: M9 live vaccine via water

12 Weeks: Autogenous vaccine

22 Weeks: M9 live vaccine by wing web

22 weeks: SRP (Siderophore receptor & Porin) vaccine including Pasteurella and E.coli

26 Weeks: SRP (Siderophore receptor & Porin) vaccine including Pasteurella and E.coli



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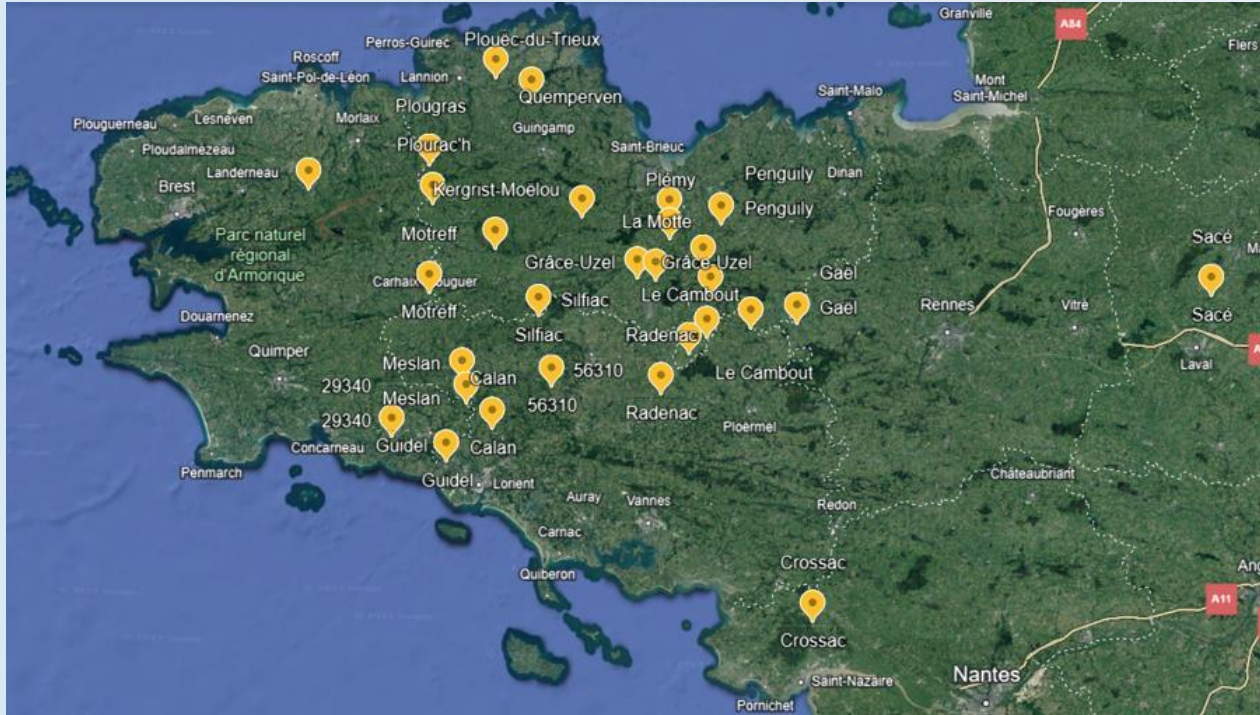


Fowl Cholera in the last 2 years (France)

- A major Fowl Cholera outbreak has occurred across Brittany since late 2022 and is still ongoing.
 - Turkey breeder flocks in all 4 different PS operations affected
 - 80 -100 cases in the past 2 years.
 - Only a few cases in commercial turkeys
- French breeder flocks historically vaccinated with autogenous vaccines or with Landavax as sporadic cases of FC were experienced.
 - These vaccines proved to be completely ineffective in the latest outbreaks.
 - The vast majority of cases have occurred in laying flocks with only a very few cases in rear.



Brittany outbreaks – first 6 months



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Example of Fowl Cholera case from January 2023 (France)

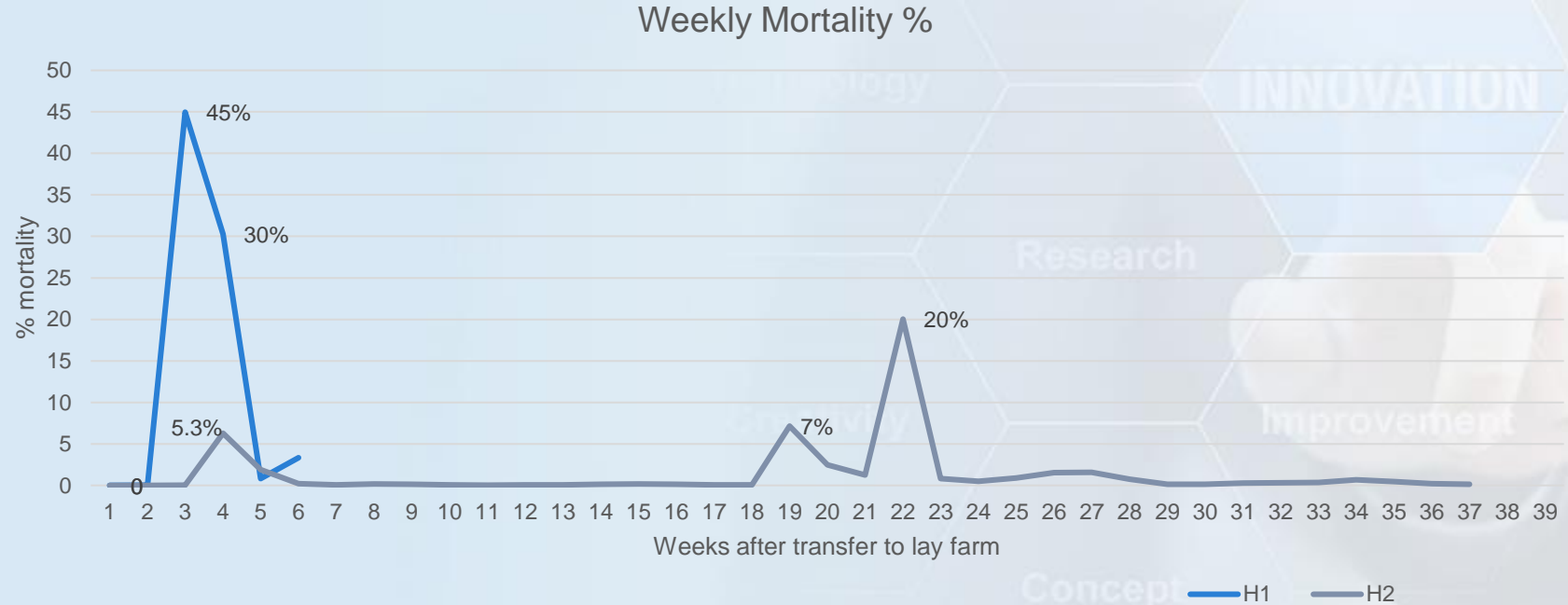
- Breeder flock of 2600 hens transferred
- 19 days after transfer mortality commenced
- Flock treated with Amoxicillin on 10th(in water)
- Flock was vaccinated with 2 doses of autogenous vaccine
- Flock was depleted

- There was also a second flock on the farm (2566 hens) reared in the same airspace as these birds. Mortality commenced 2-3 days later.
- Treatment with Tulissen (Tulathromycin) injection and Doxycycline (in water).
- Very different outcome in this flock.

Date	Mortality	% Mort	Cumulative mortality
06-Feb	2	0.08%	0.08%
07-Feb	1	0.04%	0.12%
08-Feb	0	0.00%	0.12%
09-Feb	0	0.00%	0.12%
10-Feb	26	1.00%	1.12%
11-Feb	139	5.35%	6.46%
12-Feb	1001	38.50%	44.96%
13-Feb	100	3.85%	48.81%
14-Feb	200	7.69%	56.50%
15-Feb	440	16.92%	73.42%
16-Feb	38	1.46%	74.88%
17-Feb	9	0.35%	75.23%



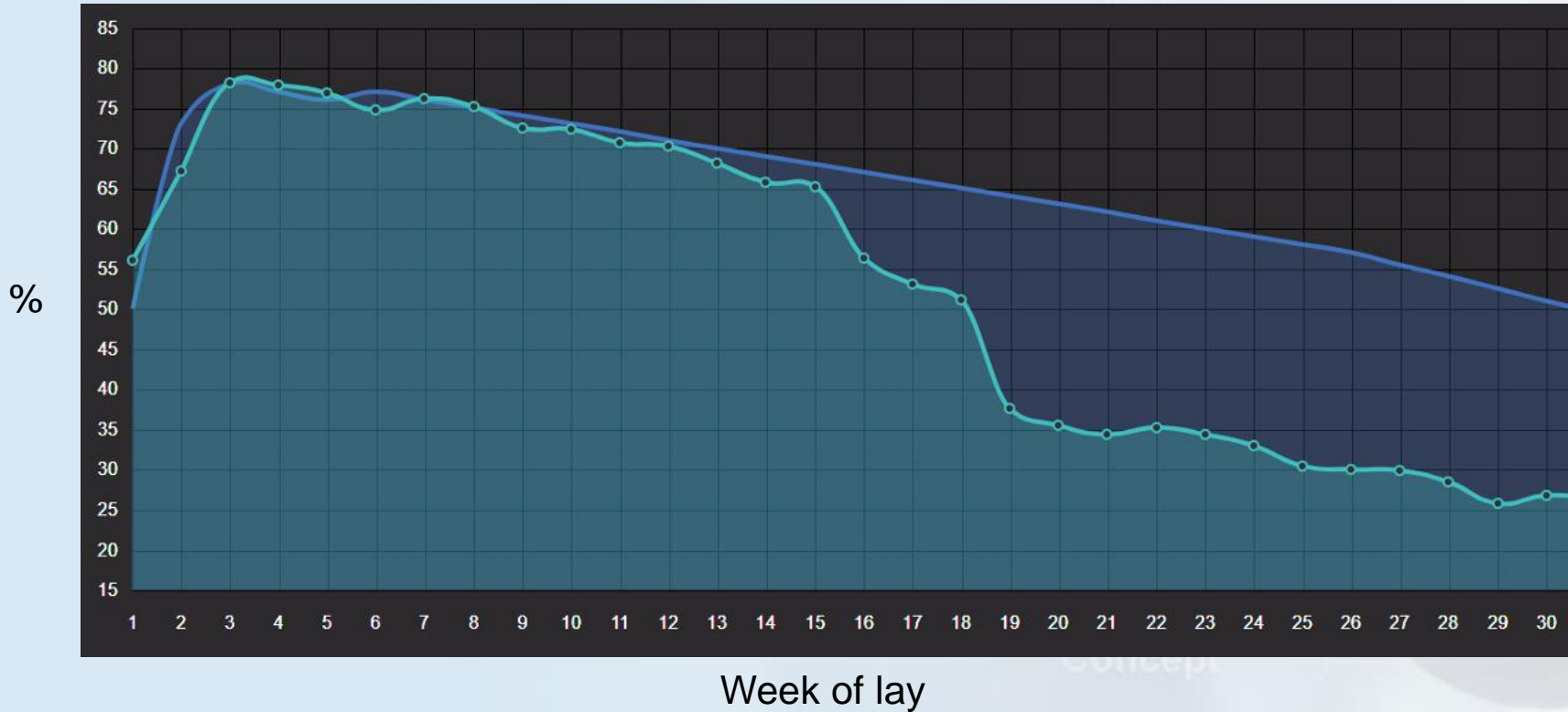
Case example: Pattern of Mortality



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Case example: Egg Production



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UK situation

- Late in 2023 and early 2024 a number of cases of Fowl Cholera occurred in the UK in a number of companies and regions.
 - Landavax vaccine and/or historical autogenous vaccines were largely ineffective.
 - Identified as Serotype 3
 - Severe mortality – breeder flocks and commercials
 - Concern we were starting to see something similar to what was occurring in France.

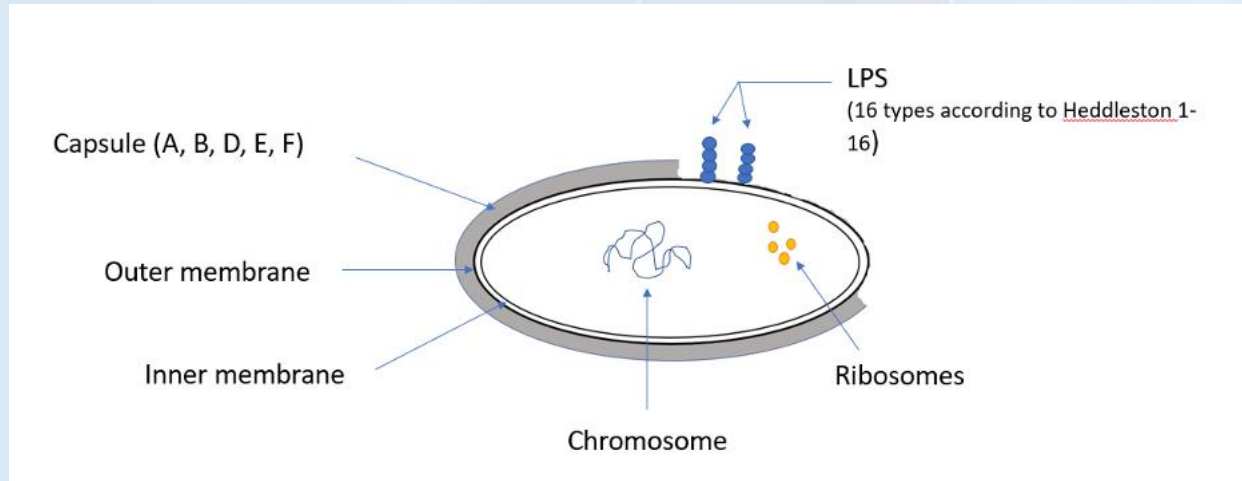
A reliable strain typing approach was required that could assist with investigating the epidemiology of these outbreaks and help to identify which strains to use in future autogenous vaccines.



Strain typing

■ Classical strain typing

- *Pasteurella multocida* strains are classified into serogroups (A, B, D, E and F) based on capsule antigens (Carter)
- further classified into 16 serotypes (1–16) based primarily on somatic lipopolysaccharide antigens using the Heddlestone scheme.
- E.g. F3; A3



Strain typing

■ PCR

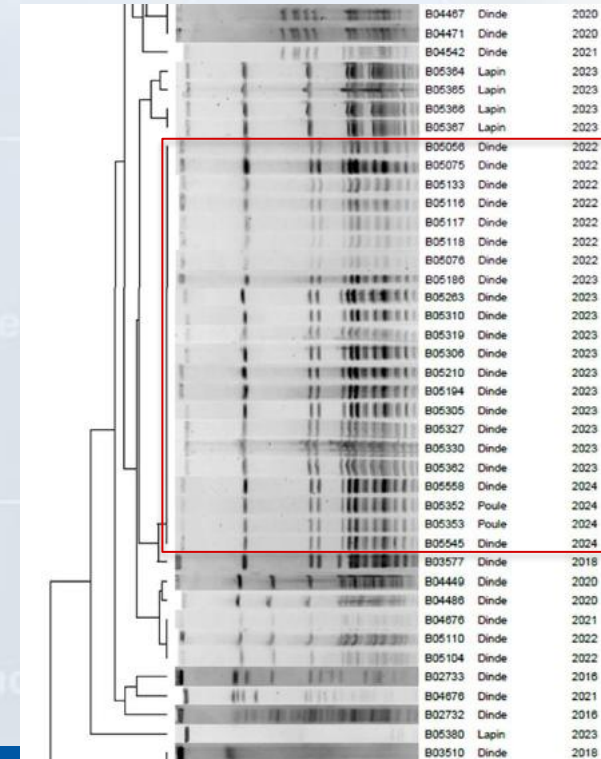
- A specific multiplex capsular PCR assay was developed that allows for rapid and specific capsular typing (Townsend et al., 2001). This assay can be used in place of the traditional capsular serotyping assay or the non-serological methods.
- An LPS multiplex PCR developed by Harper et al. (2015) targets the LPS outer core biosynthesis locus and recognises eight LPS genotypes – termed L1 to L8. The original 16 Heddleston LPS serovar reference strains are assigned as follows to the eight LPS genotypes: serovars 1 and 14 – LPS L1; serovars 2 and 5 – LPS L2; serovars 3 and 4 – LPS L3; serovars 6 and 7 – LPS L4; serovar 9 – LPS L5; serovars 10, 11, 12 and 15 – LPS L6; serovars 8 and 13 – LPS L7; serovar 16 – LPS L8.



Strain typing methods that have been used in the French outbreak: PFGE

- PFGE – Pulse field gel electrophoresis
- 3 different enzymes were used to determine if these could help differentiate between the isolates from the current outbreak cluster.
- This example is using the *SmaI* enzyme but the others had identical results.
- Unable to differentiate at all between recent turkey breeder isolates.

Gérard Lévêque, Hendrix Genetics

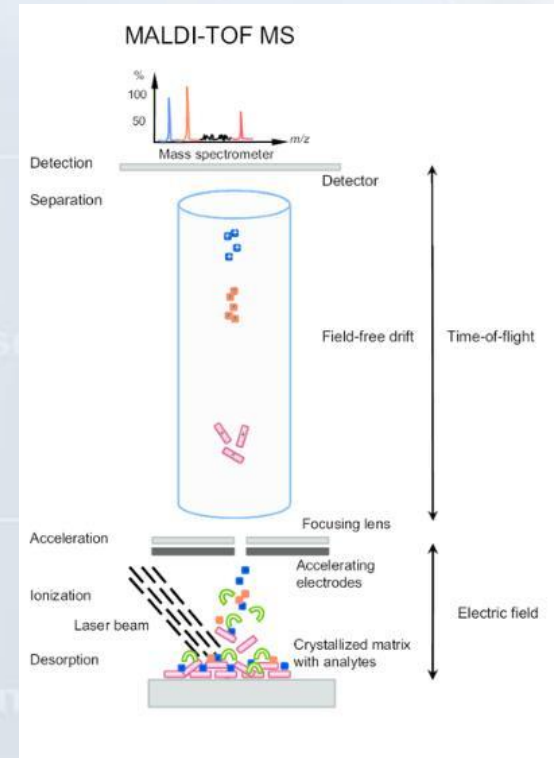


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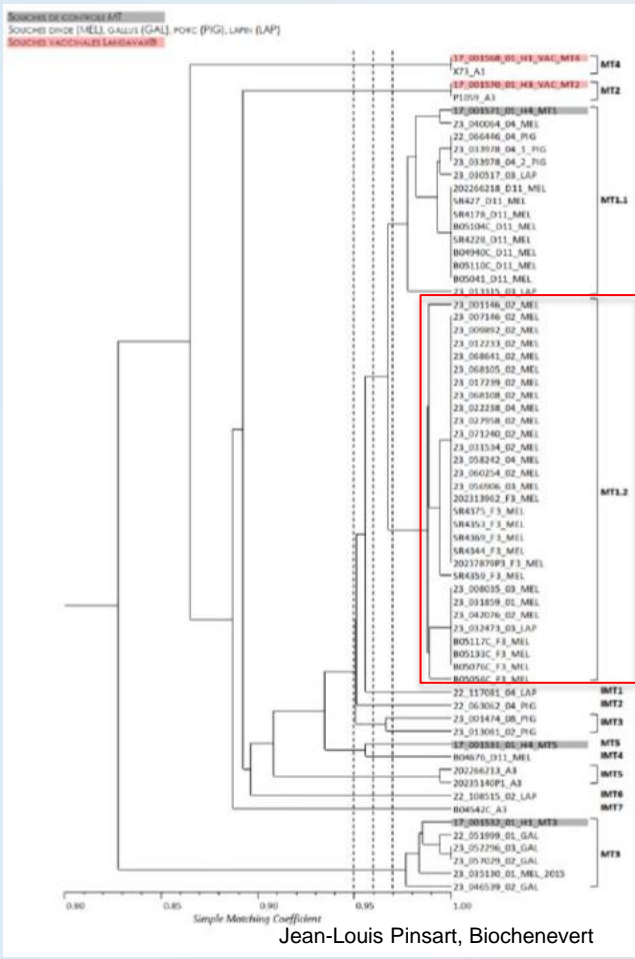


Strain typing methods that had been used in the French outbreak: MALDI-TOF

- matrix-assisted laser desorption/ionization – MALDI
- Time of Flight – TOF
- Laser impact causes thermal desorption of ribosomal proteins of bacteria embedded in matrix material and applied to the target plate
- Particles are ionized, separated according to their mass-to-charge ratio, and measured by determining the time it takes for the ions to travel to a detector at the end of a time-of-flight tube.
- MALDI-TOF MS can be used to obtain the protein profile of a strain. Using the protein profile, it is possible to detect phenotype-related biomarkers, such as drug-resistant strains, or strain sub-typing.



MALDI-TOF of French Pasteurella strains



Jean-Louis Pinsart, Biochenevert

Landavax vaccine–
Serotypes A1 & A3

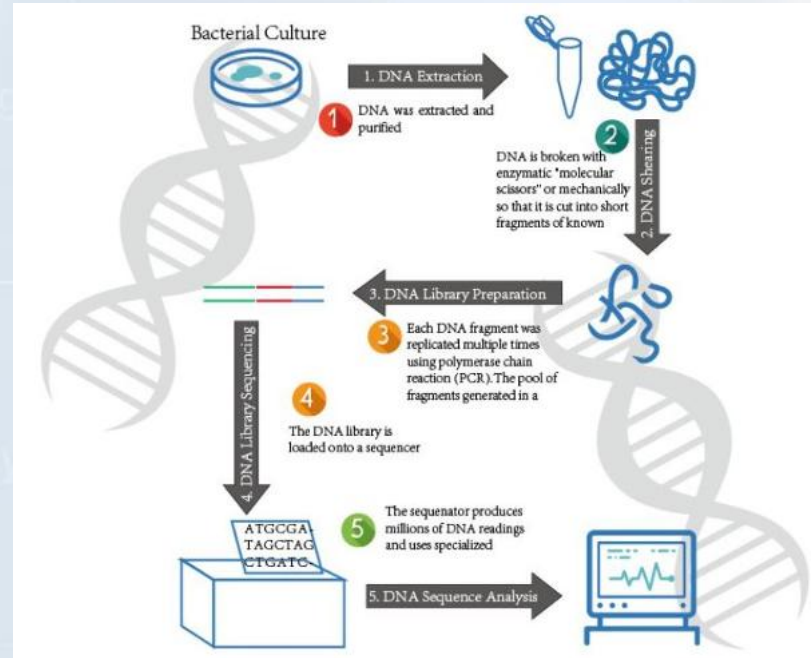
All current F3
serotype isolates fall
into MT1,2 Group



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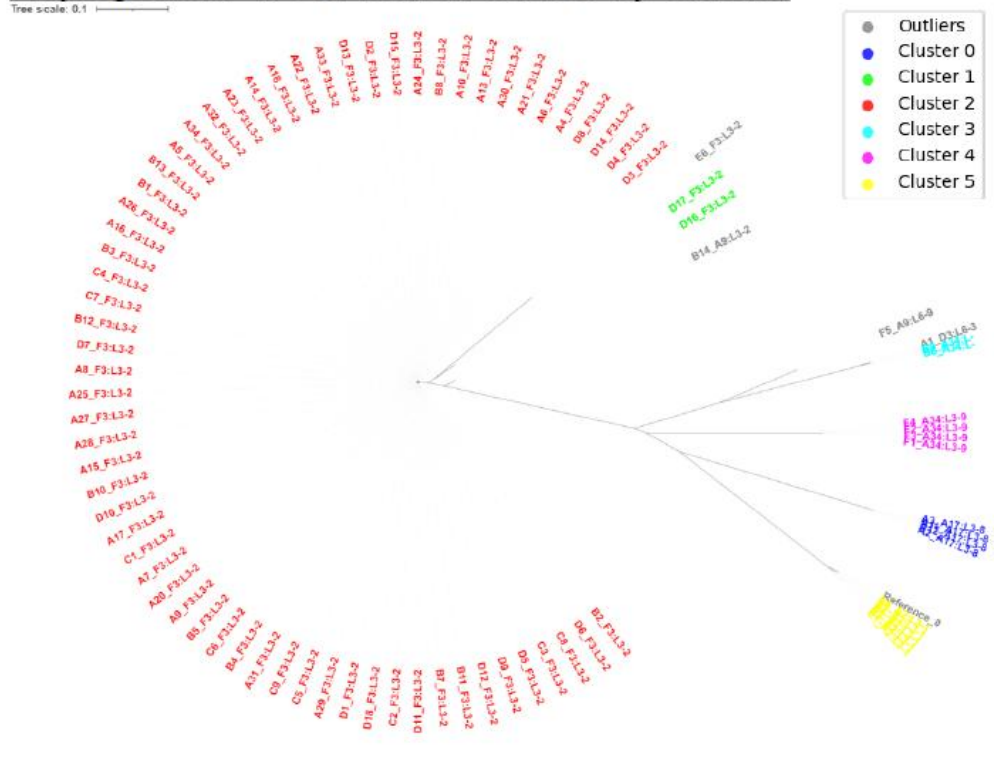
Strain typing methods that have been used in the French outbreak: WGS

- Whole Genome Sequencing – WGS
- Molecular epidemiology – looking to see if identifying changes in the genome could help determine the epidemiology of the outbreak and help determine the risk factors.
- WGS outputs
 - Predicted Serotype
 - MLST
 - SNP matrix
 - Phylogenetic trees
 - AMR genes
 - Virulence genes



Rectangular Phylogenetic tree based on SNPs

Phylogenetic Tree: Colour Coded by Cluster



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WGS: Serotype and MLST

Serotype	Number of isolates	ST
F3:L3,2	67 (66 from France, 1 UK)	179 (UK), 9 (France)
D3:L6,3	1 (France)	11
A9:L6,9	1 (UK)	10
A9:L3,2	1 (France)	12
A34:L3,9	4 (UK)	23
A34:L	2 (France)	11
A2:L3,1	5 (UK)	8
A17:L3,8	4 (France)	128



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Traditional serotyping format	Serotyping WGS format	MLST	Site Isolate ID	Date of isolation	B6	B9	F3	F4	F1	F2	E1	E2	E3	E4	E5
A:L	A34:L	11	B6	13-03-2023	0	11	20987	20934	21005	20943	25042	25046	25040	25035	25036
A:L	A34:L	11	B9	not supplied	11	0	20939	20888	20954	20901	24886	24888	24883	24878	24880
A:3	A34:L3,9	23	F3	08-02-2024	20987	20939	0	16	10	11	21322	21327	21319	21321	21322
A:3	A34:L3,9	23	F4	18-03-2024	20934	20888	16	0	18	13	21252	21258	21248	21252	21251
A:3	A34:L3,9	23	F1	28-09-2023	21005	20954	10	18	0	9	21343	21349	21338	21343	21341
A:3	A34:L3,9	23	F2	22-12-2023	20943	20901	11	13	9	0	21241	21244	21236	21236	21237
A:3	A2:L3,1	8	E1	21-01-2024	25042	24886	21322	21252	21343	21241	0	8	6	3	4
A:3	A2:L3,1	8	E2	07-03-2024	25046	24888	21327	21258	21349	21244	8	0	10	6	6
A:3	A2:L3,1	8	E3	08-03-2024	25040	24883	21319	21248	21338	21236	6	10	0	5	5
A:3	A2:L3,1	8	E4	27-10-2023	25035	24878	21321	21252	21343	21236	3	6	5	0	2
A:3	A2:L3,1	8	E5	04-09-2024	25036	24880	21322	21251	21341	21237	4	6	5	2	0

Concept



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Aviagen
turkeys

Nicholas
N.P.T.

Strain typing- outcomes

- Serotyping to Heddleston LPS serotype only e.g. serotype 3 is not sufficient and even when we have both Carter capsule serogroup and LPS serotype they can be very different.
 - E.g. UK F3 and French F3 – different MLST 179 vs 9 and differ by >9000 SNPs.
- All the strain typing methodologies (PFGE, MALDI-TOF and WGS) are able to readily distinguish between the main clusters.
 - Maldi-Tof has the advantage of being very quick and a lot cheaper but WGS gives more outputs and comparable data.
- The aim of carrying out the WGS was to determine if we could identify “transmission clusters”. This is groups of cases where direct transmission would have occurred between the cases.
- The SNP differences in the results presented were in the non-core genomic elements – some further work is going to be done using a different WGS platform to see if this is consistent.
- The SNP cut off to differentiate individual strains can be varied– the level used differs between bacterial species and the outbreak being investigated.
 - Increasing SNP cut-off may allow identification of transmission events that would otherwise be missed but will reduce specificity.



Strain typing- outcomes

- The outcome of the WGS strain typing in the main French outbreak cluster shows almost as much variation within cases as there is between cases.
 - This suggests these are all the same strain and that there is direct transmission between these cases or in some instances potential carry over on the site at turnaround.
- Given that these cases have occurred over a 2 year period and the bacteria have been exposed to major selection pressure through intensive antibiotic usage and vaccination, it is remarkable how little difference there is between the strains over a long time period and large geographical area.
 - Is this strain more transmissible? Is it better able to survive in the environment.
- Most likely routes of transmission are personnel movement (AI teams top of the list), dead bird collection service and bird transport.
 - A number of Broiler breeder cases have same strain – links?
 - A major risk factor is maintaining these infected flocks in lay which significantly increases the likelihood of transmission.
 - The situation appears to be improving!



Thank you for your attention.

Any questions?



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