Veterinary Medicines Directorate

Antimicrobial resistance (AMR) surveillance in animals in the UK

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AMR surveillance: two types

Sick animals

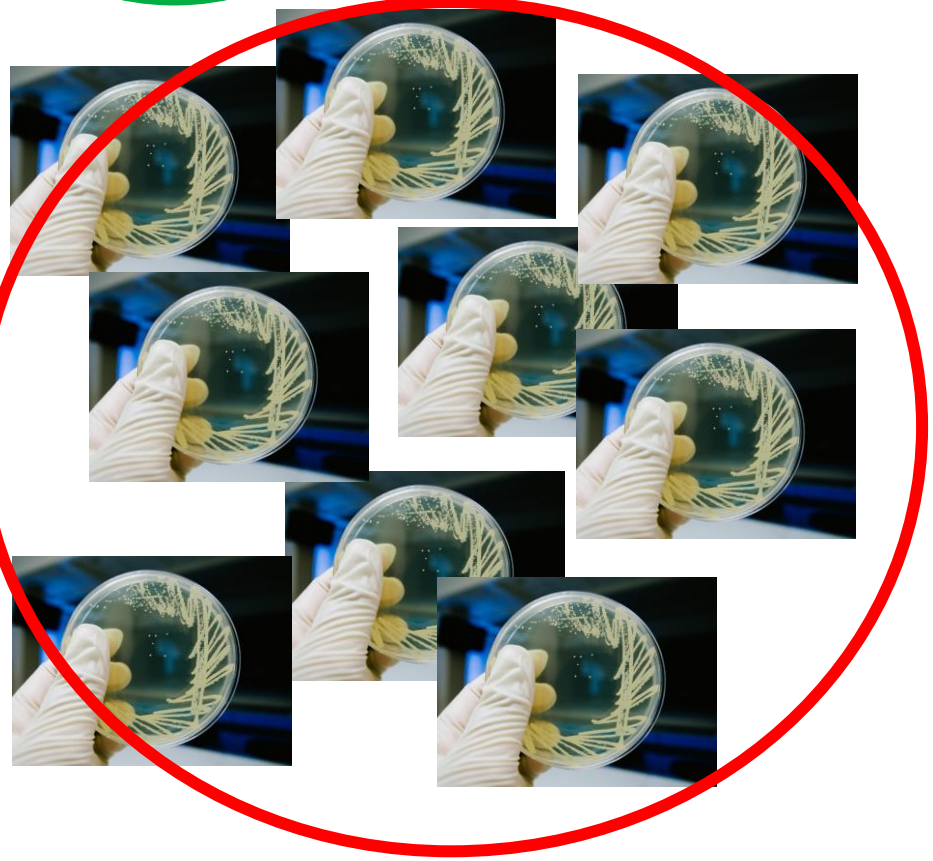
Healthy animals

AMR surveillance: sick animals

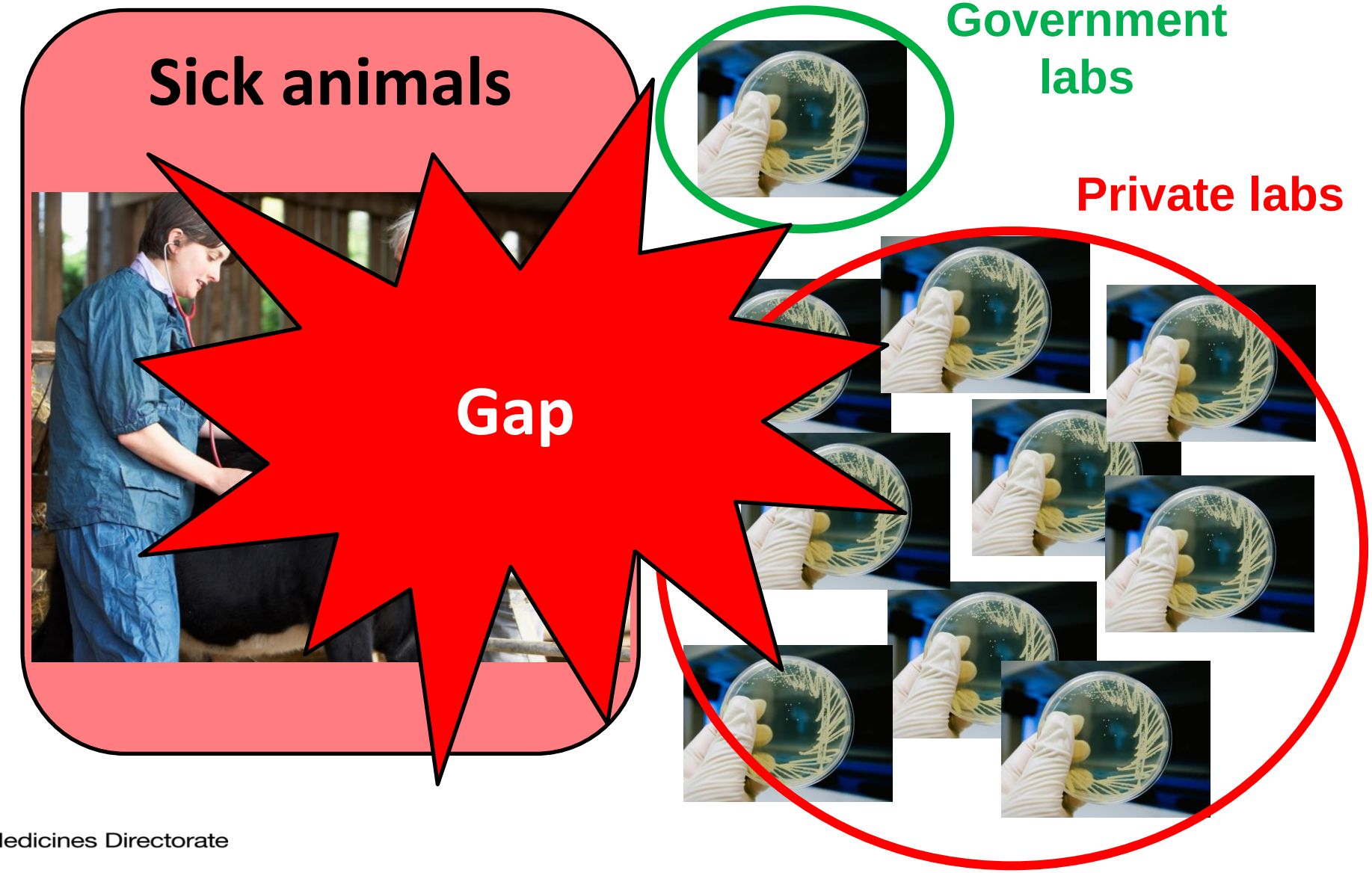
Sick animals

Government labs

Private labs



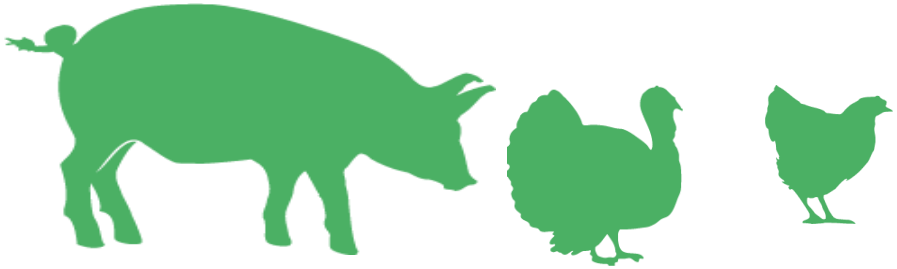
AMR surveillance: sick animals



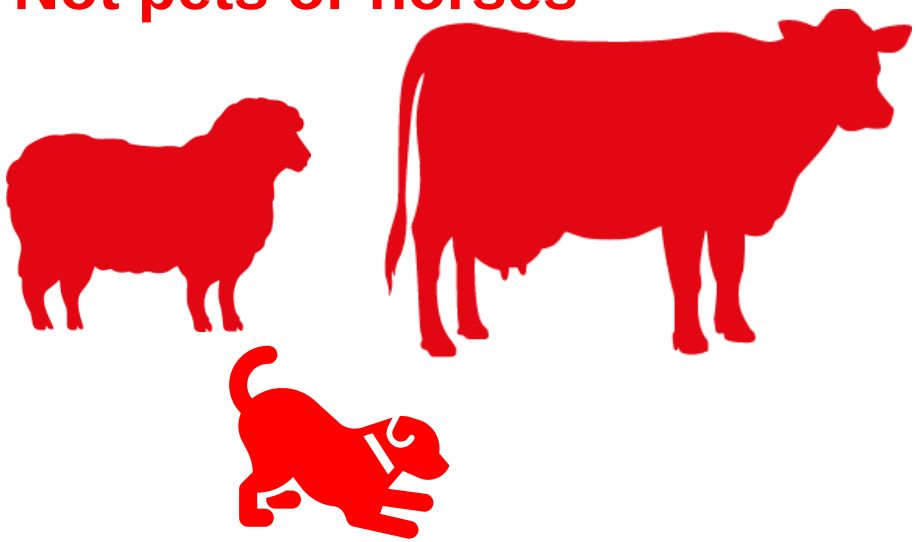
AMR surveillance: healthy animals

Healthy animals

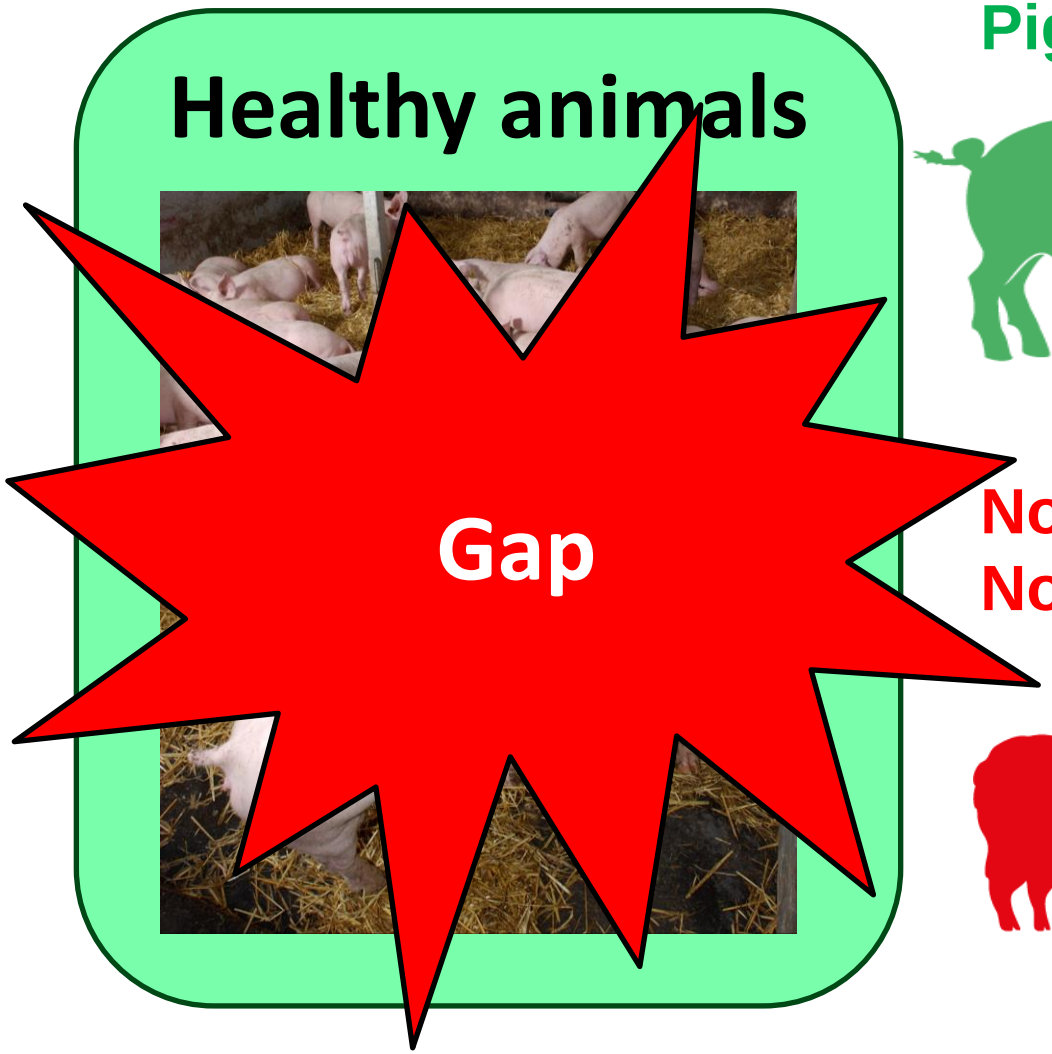
Pigs and poultry only



Not sheep or cattle
Not pets or horses



AMR surveillance: healthy animals



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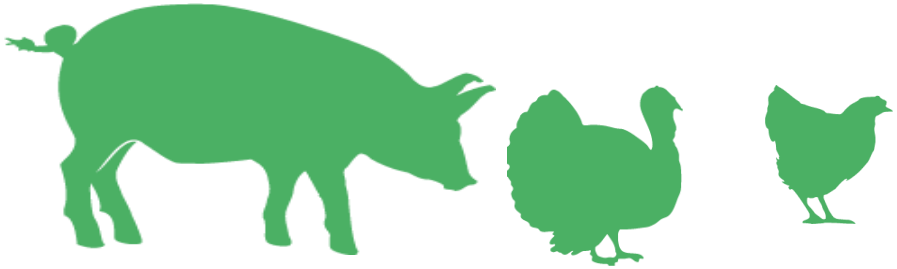

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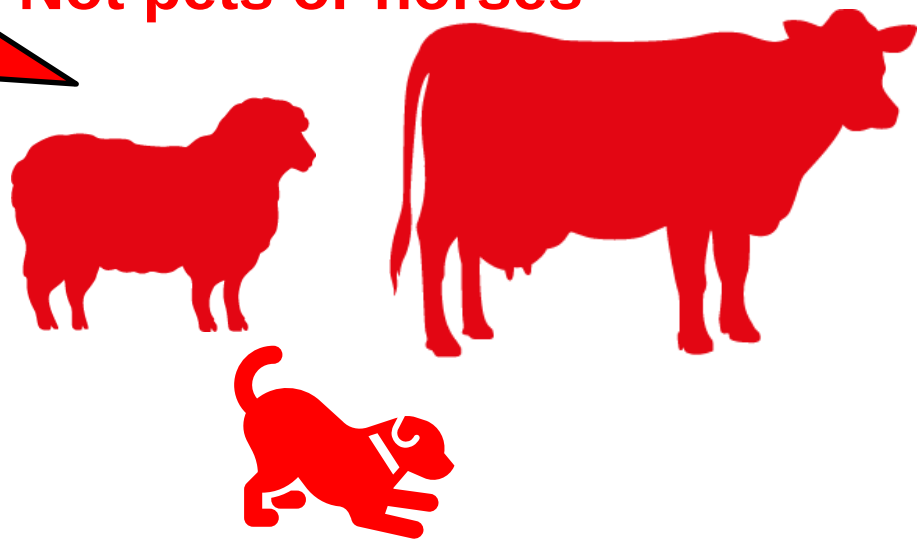


AMR surveillance: reporting

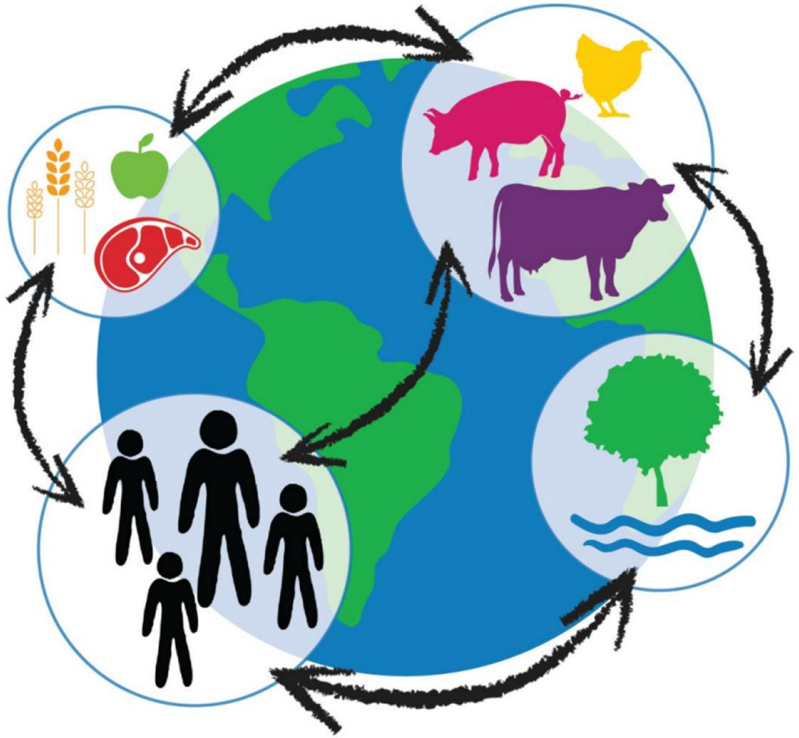
WS2b2. AMR Monitoring in abattoirs in the United Kingdom

Declan Power, Georgia Milbourne, Louise Chiverton, John Rodgers

Animal & Plant Health Agency

Background – AMR in Sheep

- Ruminant farming significant sector in UK
  - £1.5bn (2021) [1]
- Large exporter of lamb and mutton
- Previously been implication on human health – *Salmonella* outbreak related to consumption of lamb
- No routine surveillance in UK for AMR in sheep
- Limited surveillance data from UK in a one health context



PATH-SAFE Aim

Take a novel, One Health approach to AMR surveillance in sheep to assess the value of AMR surveillance data.

Provide contemporary data to update the risk assessment for both AMR and foodborne bacterial zoonoses associated with sheep meat production in the UK.

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[1] https://www.gov.uk/government/statistical-data-sets/structure-of-the-agricultural-industry-in-england-and-the-uk-at-june

Workflow Overview

Sample Collection	Microbiology	Minimum inhibitory concentration	Whole Genome Sequencing	Analysis & Reporting

• Abattoirs across England and Wales
• 30% representative coverage of annual sheep throughput
• FSA staff collect caecal contents
	• Isolate target species
	  • *Campylobacter* spp.
	  • *Enterococcus* spp.
	  • *Escherichia coli*
	• Cryopreservation
		• AMR Phenotype	• AMR genotype	• Phenotype/Genotype Analysis
				• Prevalence analysis
				• Write up

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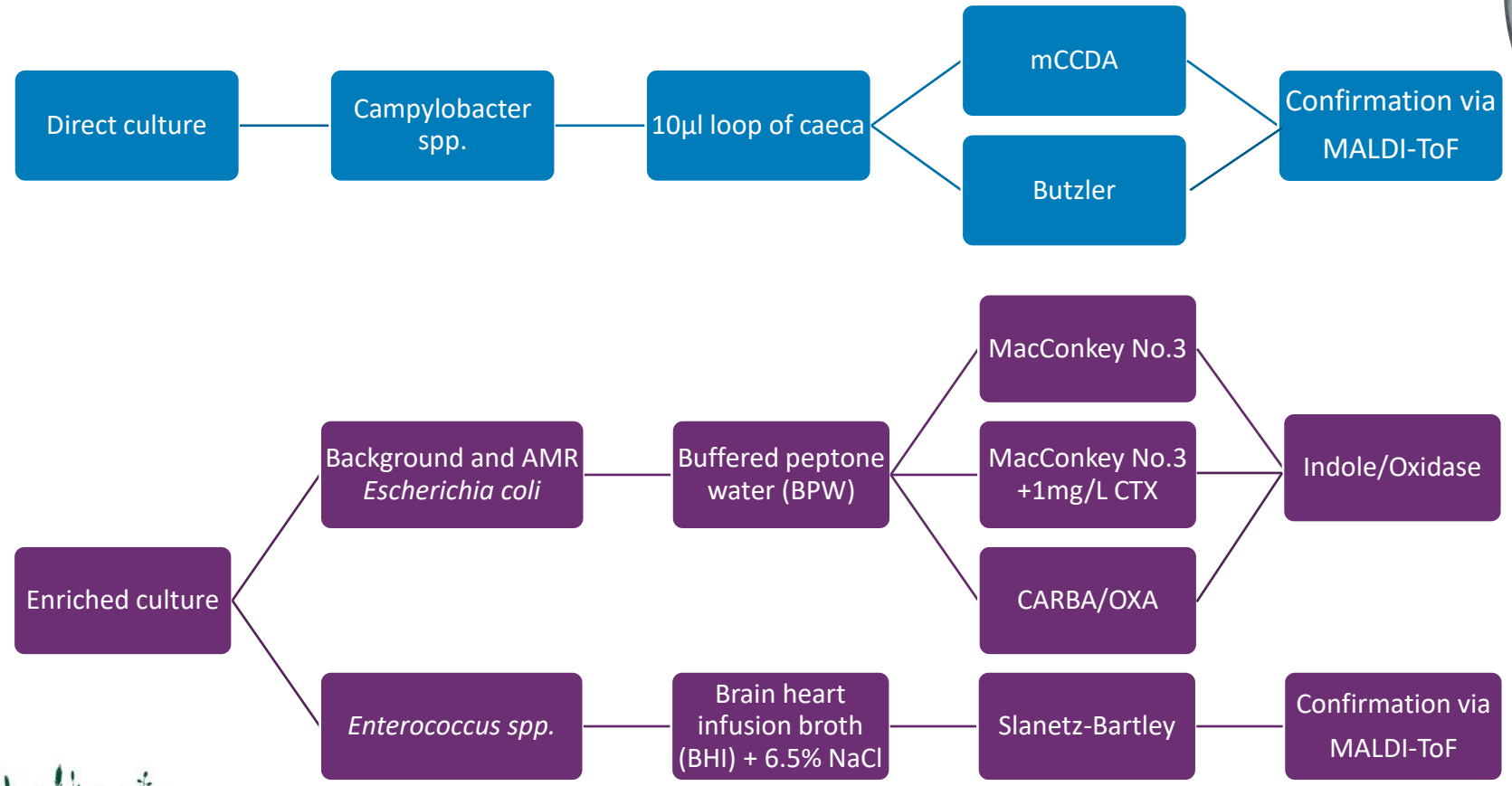
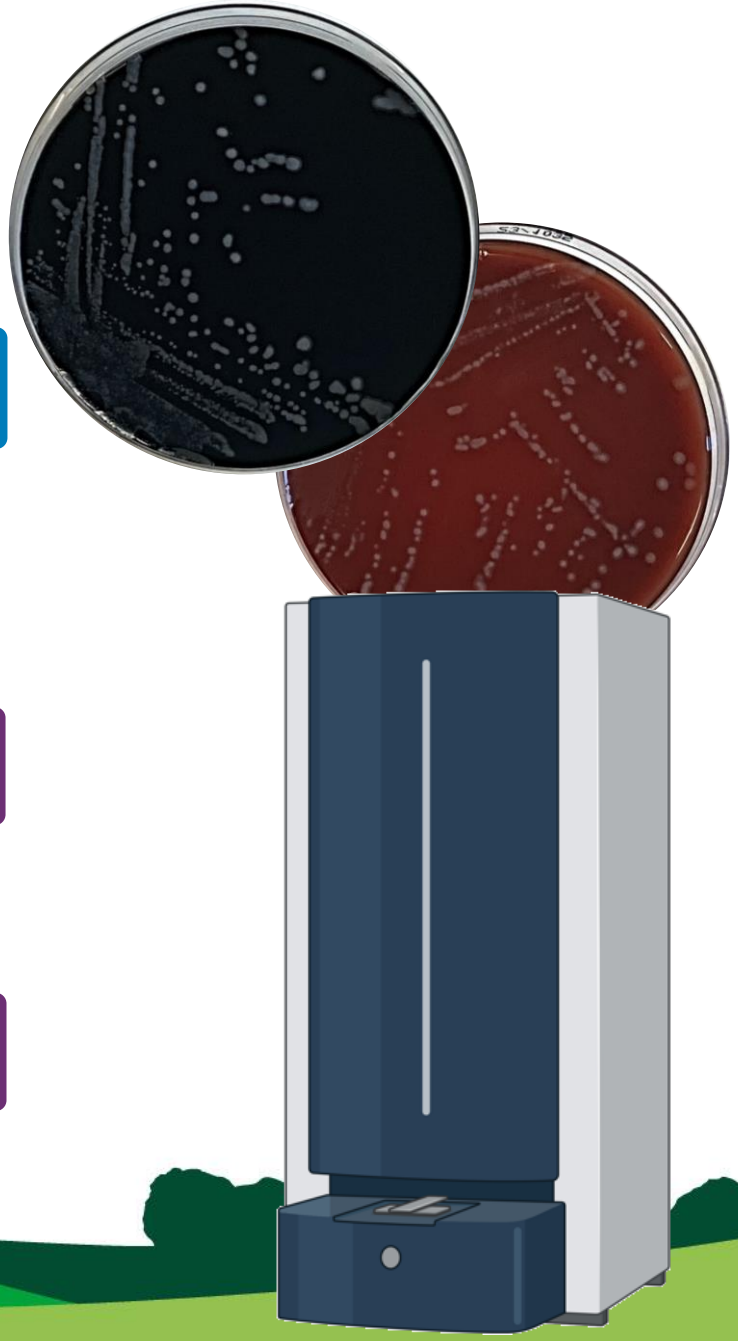
Sample collection

- Sampling kits sent out to abattoirs by APHA Weybridge
- Pre-labelled with reference IDs
- Caecal contents collected by FSA staff from healthy lambs/ ewes at slaughter
- Sampling numbers representative of abattoir throughput
- Samples shipped (chilled) within 48hrs of collection
- Samples received February - October 2023 (~1200)

Lab methods – Microbiology





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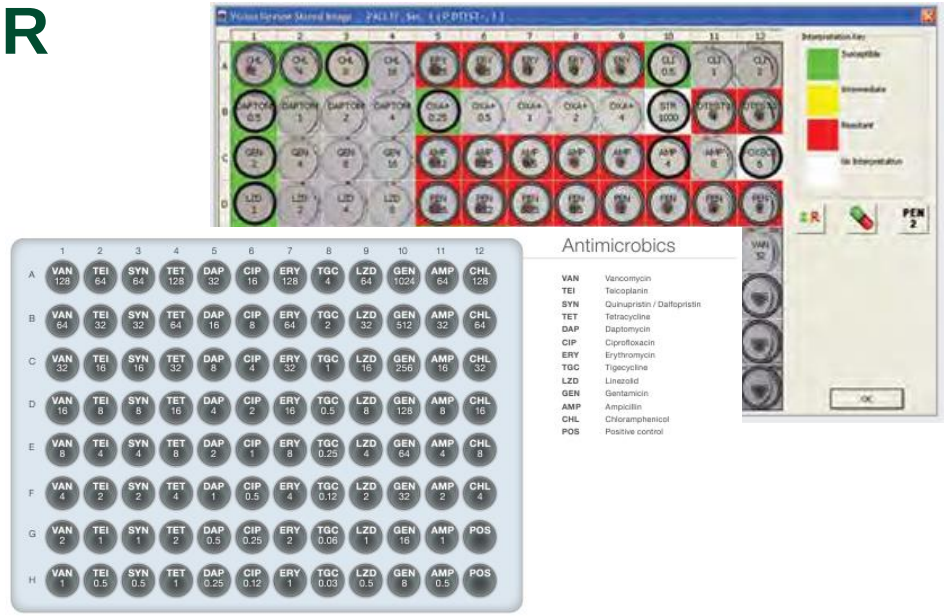
Minimum Inhibitory Concentration (MIC) – AMR Phenotype



Isolates AMR phenotype determined using the ThermoScientific™ Sensititre™ System

- Commercially produced plates with panel of Abx
- Lowest concentration to inhibit growth
- Susceptibility determined with Epidemiological cut-off value (ECOFF)

EUCAMP - *Campylobacter* spp.

EUVENC - *Enterococcus* spp.

EUVSEC2/3 - AMR (CTX)/ Background *E. coli*

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Whole Genome Sequencing

Subset of isolates sent for WGS at APHA Weybridge (*n609*)

- DNA extraction via Kingfisher
- DNA sequenced NextSeq

Illumina
Short-read sequencing

Kraken
Read classification and bacterial identification

Unicycler
De novo assembly

mlst
Multi Locus Sequence Typing

APHA SeqFinder + **Abricate**
AMR genotyping (>2700 AMR genes) + Plasmid replicons

Genotype/Phenotype (MIC) correlation

Quality control at each step

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Wastewater/ Environmental Sampling

- Current AMR survey methods include caecal and swab samples
- More passive methods of surveillance may be easier to implement in the long-run.
- However, their performance is as yet un-proven
- Determine the viability of sampling
    - Abattoir environment swabs
    - Moore swabs in floor drainage
    - Collection of wastewater
- Pilot to explore sampling methods to augment or replace existing approaches for monitoring AMR in the food production system.
    - Two abattoirs of different species

Outcomes

- Provides a surveillance baseline (for potential zoonotic campy in sheep) a previously unexplored One Health compartment.
- Prevalence baseline of AMR in organisms recovered
- Used to inform and evaluate the risks for zoonoses and AMR transmission between sheep and the consumer
- Supports the UK National Action Plan for the control of AMR.
- Data collected and isolates from this sheep survey will be archived and creates a valuable resource for future work

Future directions

- Continuation of AMR surveillance using alternative methods
- Build upon pilot data so far with more abattoir visits
- Refine and create a protocol for environmental monitoring
- Assess feasibility for FSA staff to undertake new sampling methods
- Conduct larger pilot survey with FSA staff

Acknowledgements

- APHA Weybridge
  - Bacteriology: Natasha Boodhoo, Graham Hill
  - Epidemiology: Susie Lewis
  - Lab testing: Central sequencing unit
- Project oversight
  - Francesca Martelli
- Veterinary Medicines Directorate
  - Anju Kirby
  - Tamsin Dewé
- Food Standards Agency (PATH-SAFE)
  - Rachel Baird, Ed Haynes
- Sheep and pig industry colleagues that have facilitated in abattoir visits/ participation

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Veterinary Medicines Directorate

Food Standards Agency

Veterinary Medicines Directorate

The UK Cattle AMR survey

SRUC

WS2.3b of the PATH-SAFE programme

Food Standards Agency
food.gov.uk

Who are we?

SRUC

An Lochran, Inverness Campus

Veterinary Medicines Directorate

Food Standards Scotland
For safe food and healthy eating

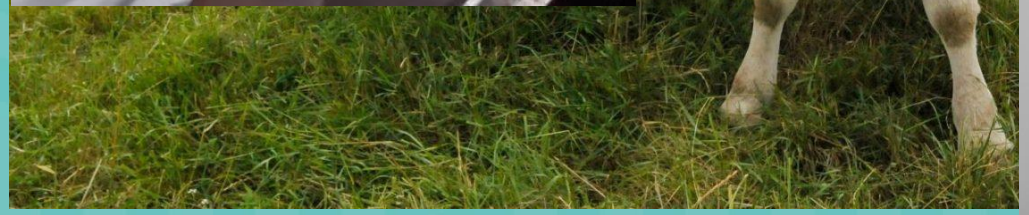
afbi Agri-Food and Biosciences Institute

Department of Agriculture, Environment and Rural Affairs
Sustainability at the heart of a living, working, active landscape valued by everyone

Food Standards Agency

The UK Cattle AMR survey

SRUC



170

350

Generic *E. coli*

Enterococci sp.

Campylobacter sp.

ESBLs

Carbapenamase

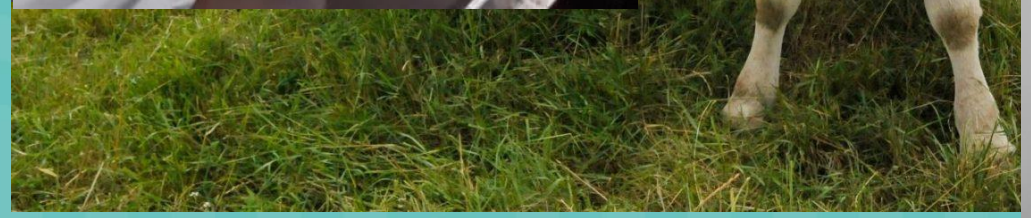
January 2023 – March 2024

Original (proposed) timeline	
January – March/April 2023	Feasibility & (possibly) set-up
April/May – October/November 2023	Operational sample collection; bacterial isolation and identification Selected isolates – MIC testing
November 2023 – December 2023	Initial reporting
January 2024 – February 2024	Further statistical analysis & final reporting
March 2024	Selected isolates to be sent for sequencing

The GB Cattle AMR survey

Veterinary Medicines Directorate

SRUC



294

?

Generic *E. coli*

Enterococci sp.

Campylobacter sp.

ESBLs

Carbapenamase

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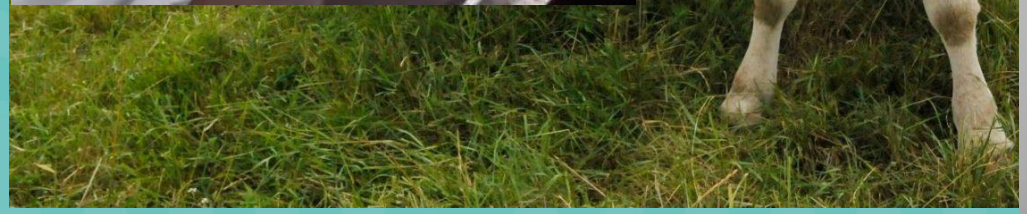
Any chance of a few more?



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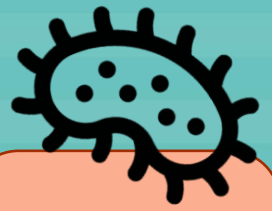

?

Generic *E. coli*

Enterococci sp.

Campylobacter sp.

ESBLs

Carbapenamase

The UK Cattle AMR survey

Veterinary Medicines Directorate

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Breaking News – EASTBio Doctoral Training programme CASE studentship awarded

Lots of lessons learnt

PILOT

For any future cattle surveys

Phenotypic susceptibility MIC

Prevalence estimates

Logistic regression – mixed model

(WGS)

WS2.3b of the PATH-SAFE programme

Food Standards Agency food.gov.uk

Thank you!

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Department of Agriculture, Environment and Rural Affairs
Sustainability at the heart of a living, working, active landscape valued by everyone

afbi Agri-Food and Biosciences Institute

Animal & Plant Health Agency

Anju Kirby & Tamsin Dewe

Rachel Baird & others

David Kyle & Hilary Glasgow

Catherine Couzens & Philip Cassidy

Catherine Fearnley, John Rodgers & team

Food Standards Agency

Food Standards Scotland
For safe food and healthy eating

Field Operations teams:
led by Richard Sharp & Steve Lomas

TopSpeed Couriers

The industry – participating premises

SRUC

Sue C Tongue
Geoff Foster

Catriona Webster
Gillian Maxwell
Jude Evans
Shannon Proctor

Madeleine Henry
Maria Costa

Roger Humphry
Jude Eze

Julie Stirling

Ian Hutchinson
Jo Baughan

Now at…

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SRUC
SCHOOL OF
VETERINARY MEDICINE

Rural and Veterinary Innovation Centre (RAVIC), Inverness Campus

PATH-SAFE WS2.4

Assessing Antimicrobial Resistance in Bulk Tank Milk: Insights from a UK Dairy Farm Survey

Manal AbuOun,
Martina Velasova, Christopher Teale

Animal &
Plant Health
Agency

Why assess AMR in Milk?

- Milk is collected from cows and combined/stored in bulk milk tanks before being collected for processing or sale.
  - efficient storage and transportation of milk from the farm to processing facilities.
- Presence of antimicrobial resistant (AMR) bacteria in bulk tank milk (raw milk direct from the cows) can result from various factors:
  - use of antimicrobials in dairy cattle.
  - environmental contamination.
  - transfer of resistant bacteria from animals to milk during the milking process.

- Bulk tank milk is subject to quality and safety standards, and it is commonly tested for various parameters:
  - bacterial count, somatic cell count, and the presence of antimicrobials or contaminants.
    - PCR testing to identify mastitis pathogens.
  - currently limited AMR testing: PCR testing of β-lactamase gene.
- Monitoring and addressing AMR in bulk tank milk could be important for ensuring food safety and addressing public health concerns related to AMR, in a One-Health framework.
  - raw milk may be consumed in parts of UK.
  - bathing milk.

National Milk Records Limited (NMR)

- Work with producers, vets, processors, government agencies, university researchers and wider dairy sector.
  - Milk recording for producers, ‘payment’ testing for processors, and health/disease testing for vets, monitoring of antimicrobial use (FarmAssit).
- Two laboratories (Wolverhampton and Glasgow) with UKAS accredited testing.
  - Extensive range of tests - from bacteriology to ELISA to genomics and GenoCells.

- Bovine Bulk Milk is collected daily by milk processor from farms and transported NMR lab for testing, including statutory purposes.

Test	Purpose
Total viable count (TVC).	Hygienic milk production
Somatic cell count (SCC)	Indirect measure of mastitis
Physical properties	Milk Quality
Residues	Food Safety
Bacteriology	Cattle health, investigation of herd and environmental issues.
Serology	Exposure to various diseases.

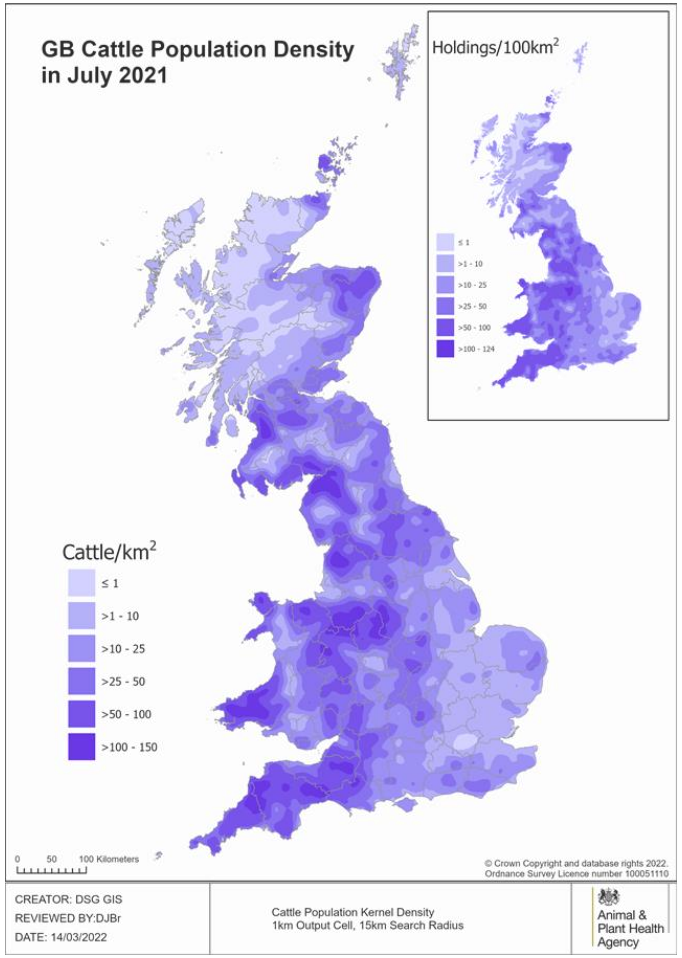
FarmAssist – working with processors to monitor antimicrobial use across their milk pool

- ✓ Seven years of data (2017 to 2023)
- ✓ Multiple processors and retail groups
- ✓ 210 + vet practices
- ✓ 1,000 + herds
- ✓ 240,000 cows

- Bulk milk can be used to investigate occurrence of bacterial mastitis pathogens, zoonotic and other organisms.

Aims of PATH-SAFE AMR in Bulk Milk

- Investigate the occurrence and dynamics of various indicator bacteria and the antimicrobial resistance they may harbour.
- Identify regional differences of indicator bacteria (anonymised by region).
- Develop and test qPCR for detection of class 1 integrons as a general marker for the prevalence of resistance in Gram-negative organisms in bulk milk.
- Sample size: 1050 bulk milk samples over 9 months.
  - January – October 2023



Overview of Bulk Milk project

Study Design	Weekly Sample collection & delivery	Microbiology	Minimal inhibitory concentration	Bacterial characterisation	Data analysis & Reporting
APHA PATH-SAFE	**National Milk Labs (NML)**	**APHA Starcross**	**APHA Starcross**	**APHA Weybridge**	**APHA Weybridge**
• Agreements • Planning • Coordination • APHA supplied collection vessels	25 Milk samples/ week	• Isolation of target bacteria • Freeze to beads	AMR phenotype of target bacteria	MALDI of *Enterococcus*	• Whole genome sequence analysis • Epidemiology analysis
	100 Milk samples/month			• DNA extraction • Sequencing of target bacteria	
				Integron PCR direct on Milk	

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AMR in Bulk Milk: Collection

- National Milk Labs (NML)
  - Permission from Arla Foods (the milk processor) to use their samples.
  - Arla foods collected samples from their producers.
  - Co-ordinated selection 100 randomly selected farms per month.
    - Proportional to NUTS1 region.
  - Collected
    - 5ml of each milk sample for microbiology.
      - ~25 x fresh samples sent to APHA Starcross weekly
    - 1ml of milk for Integron PCR.
      - Frozen samples sent monthly to APHA Weybridge.
    - Sample ID and region sent to labs in advance of samples.

  - Samples arrive weekly on Friday > refrigerated over weekend prior to testing.
    - Between January and October 2023

AMR in Bulk Milk: Microbiology

- 1ml of milk sample
  - Direct plating
    - *Klebsiella spp*, *E. coli*, *Streptococcus spp, S. aureus*
  - Enriched in
    - Buffered peptone water (BPW)
      - *E. coli*, Extended Spectrum Cephalosporins (ESC) and Carbapenem resistant Enterobacteriaceae
    - Brain heart infusion broth (BHI) + 6.5% NaCl
      - *Enterococcus spp*
    - MHB+6.5% NaCl
      - MRSA
- After enrichment, broths are plated on selective agars.
  - Following selection a target colony from each plate is purified twice on blood agar prior to archiving on -80^{o}C beads.
  - The microbiology process takes approx. 1 week.
  - Frozen beads are sent to APHA Weybridge for characterisation.

Bacterial species	plates	Isolation Agar
Streptococcus spp	Direct	Blood Edwards MacConkey
Klebsiella spp	Direct	
E. coli	Direct/Enrich	
Staphylococcus aureus	Direct	
Enterococcus faecalis *Enterococcus faecium*	Enrich	Slanetz-Bartley
MRSA	Enrich	Brilliance MRSA
Extended Spectrum Cephalosporins (ESC)	Enrich	MacConkey +1mg/L CTX
CARBA/OXA	Enrich	ChromidCARBA


Blood agar


MacConkey

Examples

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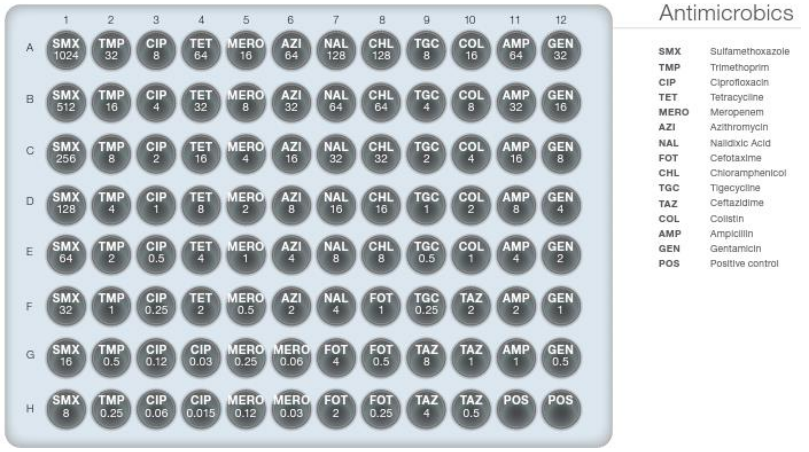
AMR in Bulk Milk: MIC characterisation

- APHA Starcross
  - Minimal Inhibitory Concentration (MIC) testing is performed on all target organisms using the Sensititre system using predefined plates.
    - MIC is the lowest concentration required of the specific antibiotic to inhibit visible *in vitro* growth.
    - Epidemiological cut-off value (ECOFF) used to determine if bacteria is susceptible or non-susceptible to an antibiotic.



Bacterial species	MIC plates	No. of antibiotics
Streptococcus spp	GBPAPHA3	15
Klebsiella spp	EUVSEC3	15
E. coli		
Enterococcus faecalis / faecium	EUVENC	12
Staphylococcus aureus	EUST2	19
Methicillin resistance *Staph aureus* (MRSA)		
ESC *E. coli*	EUVSEC2/3	14
Carbapenemase producing Enterobacteriaceae		15

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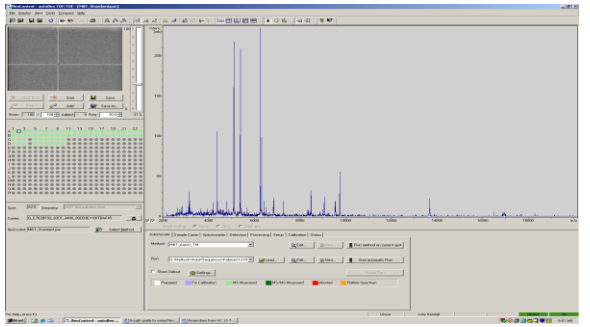
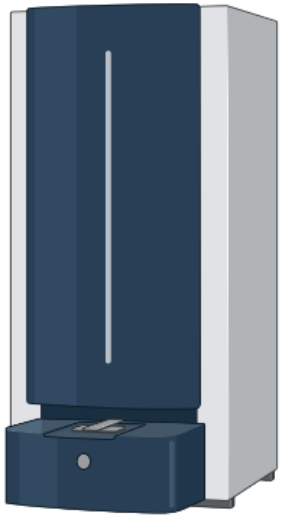
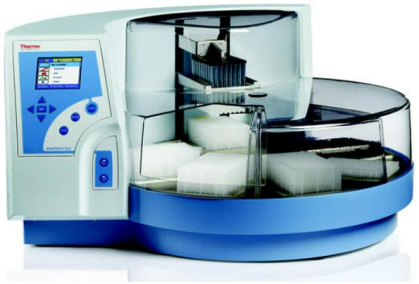
AMR in Bulk Milk: WGS Characterisation

- APHA Weybridge
  - Where identification of species cannot be achieved by culture, MALDI analysis is performed – mainly *Enterococcus* spp.





  - WGS of target bacteria
    - DNA extracted using Kingfisher.
    - DNA sequenced using NextSeq platform.
    - Sequence analyses workflow:



Illumina
Short-read sequencing

Kraken
Read classification and bacterial identification

Unicycler
De novo assembly

mlst
Multi Locus Sequence Typing

APHA SeqFinder + **Abricate**
AMR genotyping (>2700 AMR genes) + Plasmid replicons

Genotype/Phenotype (MIC) correlation

Quality control at each step

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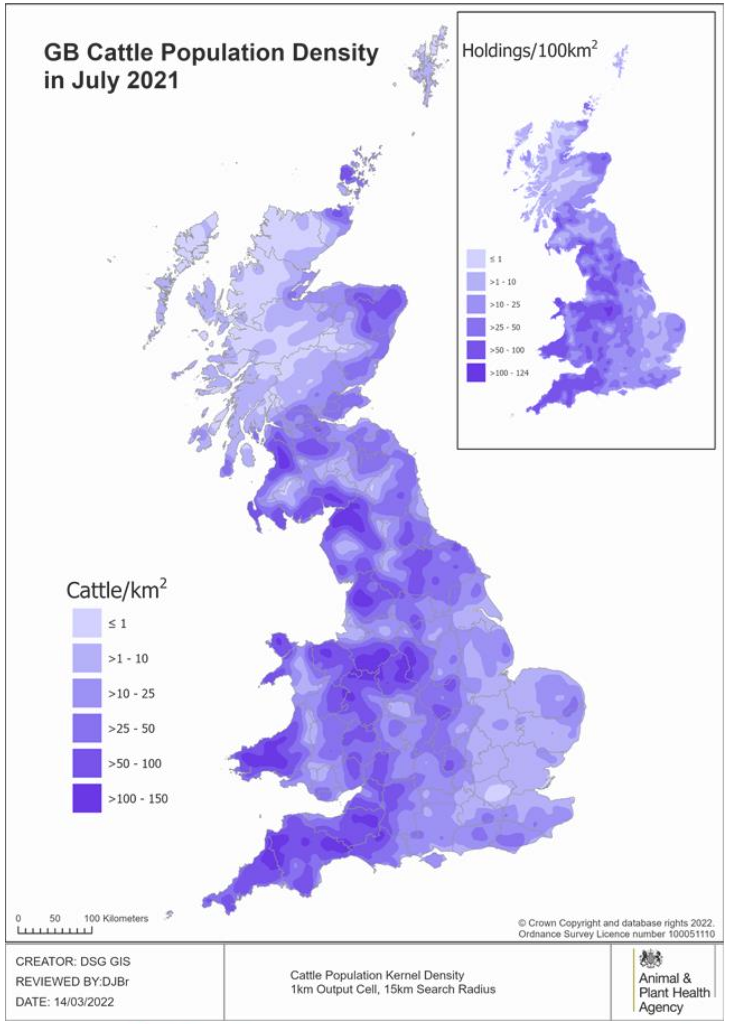
Epidemiology Data analysis

- Farms were stratified by NUTS1 region within GB (10 regions incl. Scotland & Wales).
- From each region, a representative sample was taken proportional to the size of its dairy cattle population.

- *Analytical Objectives*:
  - Estimate the prevalence of AMR pathogens (*E. coli*, ESBL-producing *E. coli*, *Enterococcus*, and *S. aureus*) and AMR genes in the GB dairy cattle population using bulk milk samples.
  - Compare regional variation in the prevalence of AMR resistant pathogens/genes descriptively.



Acknowledgements

- Arla Foods & NML for the samples
  - NML: Manmeet Bansal, Ashu Bassan, Eamon Watson
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  - Laura Biddle, Holly Slade, Jeremy Chanter, Henry Mcilroy, Thomas Saunders, Theresa Carson
- APHA Weybridge
  - Bacteriology: Natasha Boodhoo, Graham Hill, James Coates, John Rodgers, Nick Duggett
  - Epidemiology: Monty Payne, Chloe Manning, Martina Velasova,
  - Lab testing: Central sequencing unit
- Project oversight
  - Christopher Teale, Richard Smith, Martina Velasova, Muna Anjum, Francesca Martelli
- Veterinary Medicines Directorate
  - Anju Kirby, Tamsin Dewé
- Food Standards Agency (PATH-SAFE)
  - Rachel Baird, Ed Haynes

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Veterinary Medicines Directorate

Food Standards Agency

PATH-SAFE WS2.5

Establishing a Baseline for Antimicrobial Resistance in Animal Feed

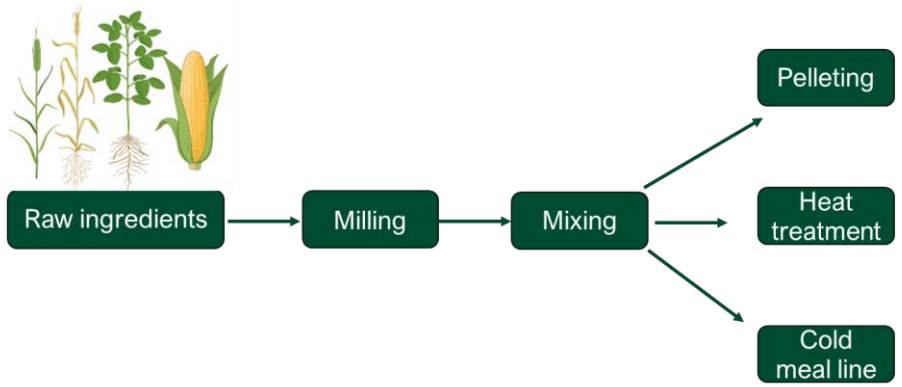
Manal AbuOun,
Martina Velasova, Christopher Teale

Animal & Plant Health Agency

Why assess Animal Feed for AMR?

- Animal feed ingredients are traded globally and could represent a route of transmission of pathogens and AMR genes into the food chain.
  - UK has reduced antibiotic use in livestock by over 50% since 2014 and lower levels of AMR in animals

- Currently there is no testing for antimicrobial resistance bacteria in animal feed (imported raw ingredients or finished products).
  - Feed and feed ingredients represent a potential source of risk to animal and human health.
    - potential to affect multiple herds/ flocks if present
  - Only routine monitoring of animal feed is for *Salmonella*
  - In other countries there is limited detection of AMR.
    - Sweden and USA possible role of feed in dissemination of AMR



- Understanding the AMR load in Animal feed, to make risk-based decisions about surveillance and control measures.

Agricultural Industries Confederation (AIC)

- AIC is trade association for the agricultural sector.
  - Animal Feed - 90%
  - Crop Protection & Agronomy - 90%
  - Fertilisers (Distributors) - 95%
  - Grain & Oilseeds (Arable Marketing) - 90%
  - Seed - 80%
- Working in support of modern sustainable commercial agriculture in the UK and supports collaboration throughout the food chain.
  - Supplying UK livestock farms
    - New feeds and alternative proteins
    - Feed additives
    - Medicated feed authorisation
  - Sustainability
    - Responsible sourcing of soy and palm oil
  - Feed safety
    - Regulation
    - Animal health and welfare

Aims of PATH-SAFE AMR in Animal Feed

- Working in partnership with a UK animal feed mill manufacturing pig and poultry feed.
    - Prevalence of various indicator bacteria and AMR bacteria in raw material ingredients and finished feed product.
    - Genomic characterisation of bacterial isolates.
    - Identify risk of pathogens and AMR in the raw material ingredients and finished products.
- Sample size: 600 samples over 10 months
    - January – October 2023

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Overview of Animal Feed project

Study Design	Weekly Sample collection & delivery	Microbiology	Minimal inhibitory concentration	Bacterial characterisation	Data analysis & Reporting
APHA PATH-SAFE	**Feed Mill**	**APHA Newcastle**	**APHA Starcross**	**APHA Weybridge**	**APHA Weybridge**
• Agreements • Planning • Coordination • APHA supplied collection vessels	10x Feed Ingredients & 10x Finished Products	• Isolation of target bacteria • Freeze to beads	AMR phenotype of target bacteria	MALDI of *Enterococcus* • DNA extraction • Sequencing of target bacteria	• Whole genome sequence analysis • Epidemiology analysis

Animal & Plant Health Agency

AMR in Animal Feed: Collection

- Feed Mill
  - APHA trained mill staff to collect samples, including ways to avoid cross contamination between different sample types
  - Mill staff collect and send 25g of each selected sample type
  - Each week, 10 raw ingredients + 10 finished feed products (total of each 300)
  - Sample information sent to lab in advance of samples
  - Samples are couriered and typically arrive on Wednesdays
    - Between January and September 2023

Raw Ingredients	Finished Products
Barley	Cold line poultry pellets
Wheat	Poultry meal
Maize	Heat treated poultry pellets
High Protein Soya	Pig Pellets
Rape Meal Extract	Pig cakes
Sunflower	

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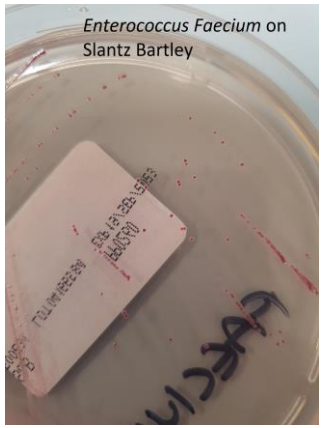
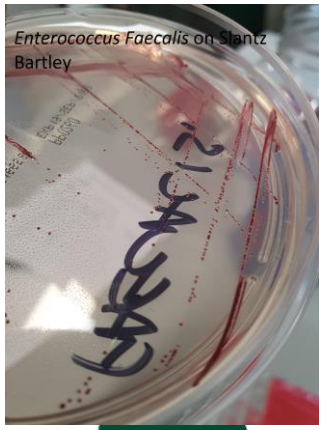
AMR in Animal Feed: Microbiology

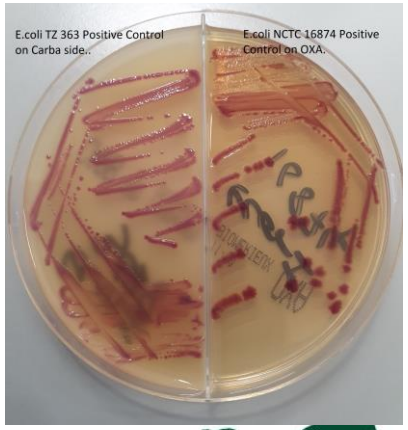
- APHA Newcastle
  - 25g of each product are enriched
    - Brain heart infusion broth (BHI)
      - Enterococcus spp.
    - Buffered peptone water (BPW)
      - *E. coli*, *Klebsiella* spp., Extended Spectrum Cephalosporins and Carbapenemase producing resistant *Enterobacteriaceae*, *Salmonella* enterica
  - After enrichment, broths are plated on selective agars
  - Following selection, a target colony from each plate is purified twice on blood agar prior to archiving on -80^{o}C beads.
  - The microbiology process takes approx. 1 week
  - Frozen beads are sent to APHA Starcross and APHA Weybridge for MIC, MALDI & WGS

Target Bacteria	Isolation Agar
E. coli	MacConkey
Klebsiella spp	MacConkey
Enterococcus faecalis *Enterococcus faecium*	Slanetz-Bartley
Extended Spectrum Cephalosporins (ESC)	MacConkey +1mg/L CTX
Carbapenemases producing *Enterobacterales*	ChromidCARBA
Salmonella enterica	MRSV > MKTT broth > XLD and Brilliant green

Examples







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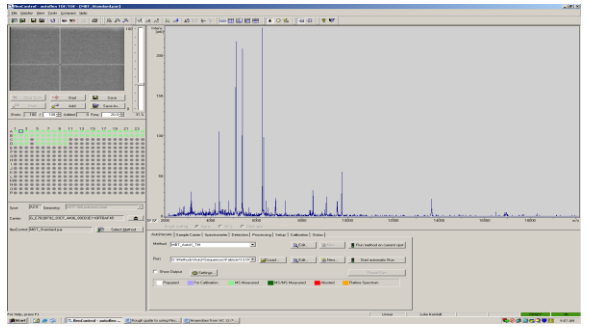
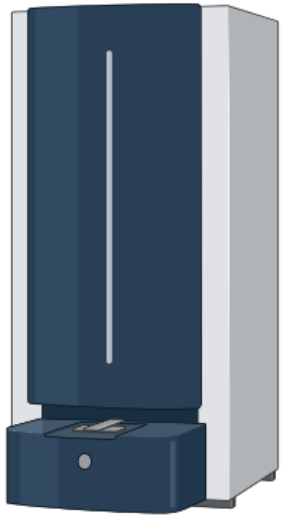
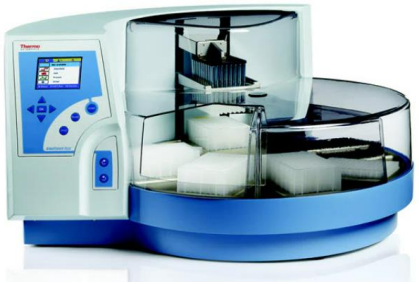
AMR in Animal Feed: WGS Characterisation

- APHA Weybridge
  - Where identification of species cannot be achieved by culture, MALDI analysis is performed – mainly *Enterococcus* spp.





  - WGS of target bacteria
    - DNA extracted using Kingfisher
    - DNA sequenced using NextSeq platform
    - Sequence analyses workflow



Illumina
Short-read sequencing

Kraken
Read classification and bacterial identification

Unicycler
De novo assembly

mlst
Multi Locus Sequence Typing

APHA SeqFinder + **Abricate**
AMR genotyping (>2700 AMR genes) + Plasmid replicons

Genotype/Phenotype (MIC) correlation

Quality control at each step

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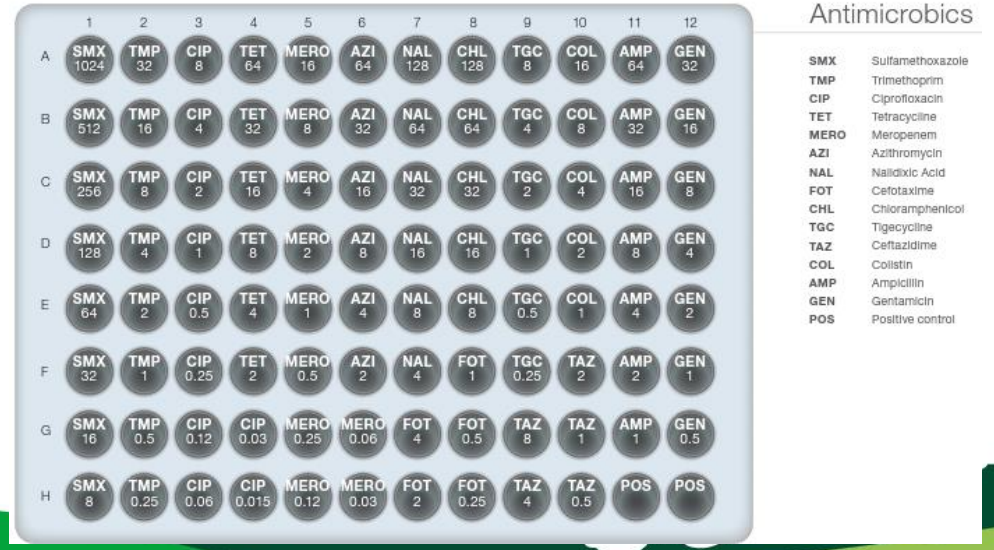
AMR in Animal Feed: MIC characterisation

- APHA Starcross
  - Minimal Inhibitory Concentration (MIC) testing is performed on all target organisms using the Sensititre system using predefined plates
    - MIC is the lowest concentration required of the specific antibiotic to inhibit visible *in vitro* growth
    - Epidemiological cut-off value (ECOFF) used to determine if bacteria is susceptible or non-susceptible to an antibiotic

Bacterial species	MIC plates	No. of antibiotics
Klebsiella spp	EUVSEC3	15
E. coli		
Salmonella		
Enterococcus faecalis / faecium	EUVENC	12
ESC *E. coli*	EUVSEC2/3	14
Carbapenemase producing Enterobacteriaceae		15



Epidemiology Data analysis

- Feed mill selected within the Agricultural Industries Confederation (AIC).
- Sample size based on volume used (raw ingredients) and produced (finished feed products) by the feed mill.
  - 300 raw feed ingredients
  - 300 finished feed product.
  - 99% confidence that AMR occurring at a prevalence of 5% will be detected in at least one sample, and 95% confidence of detecting 1% prevalence of resistance.
- *Analytical Objectives*:
  - Determine the proportion of AMR within ingredient & finished feed samples.
  - Estimate the prevalence of pathogens within ingredient & finished feed samples.

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