

Meat species carry-over occurs in meat processing plants – truth or fallacy?



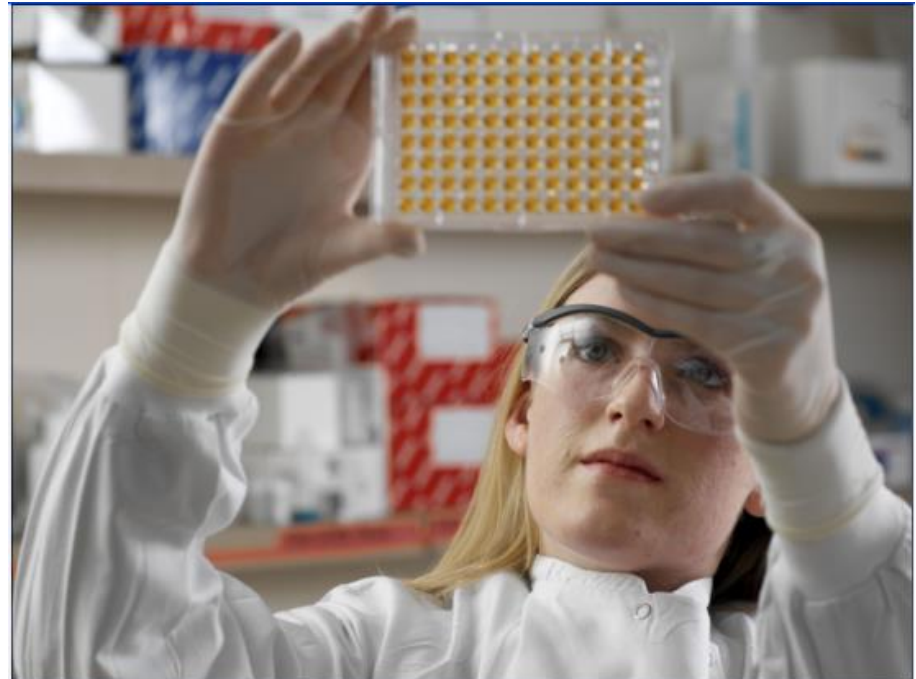
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Science
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Agenda

- Background
- Project design
- Aim
- Validation
- Phase 1
- Phase 2
- Interpretation
- Conclusions
- Acknowledgements



Background



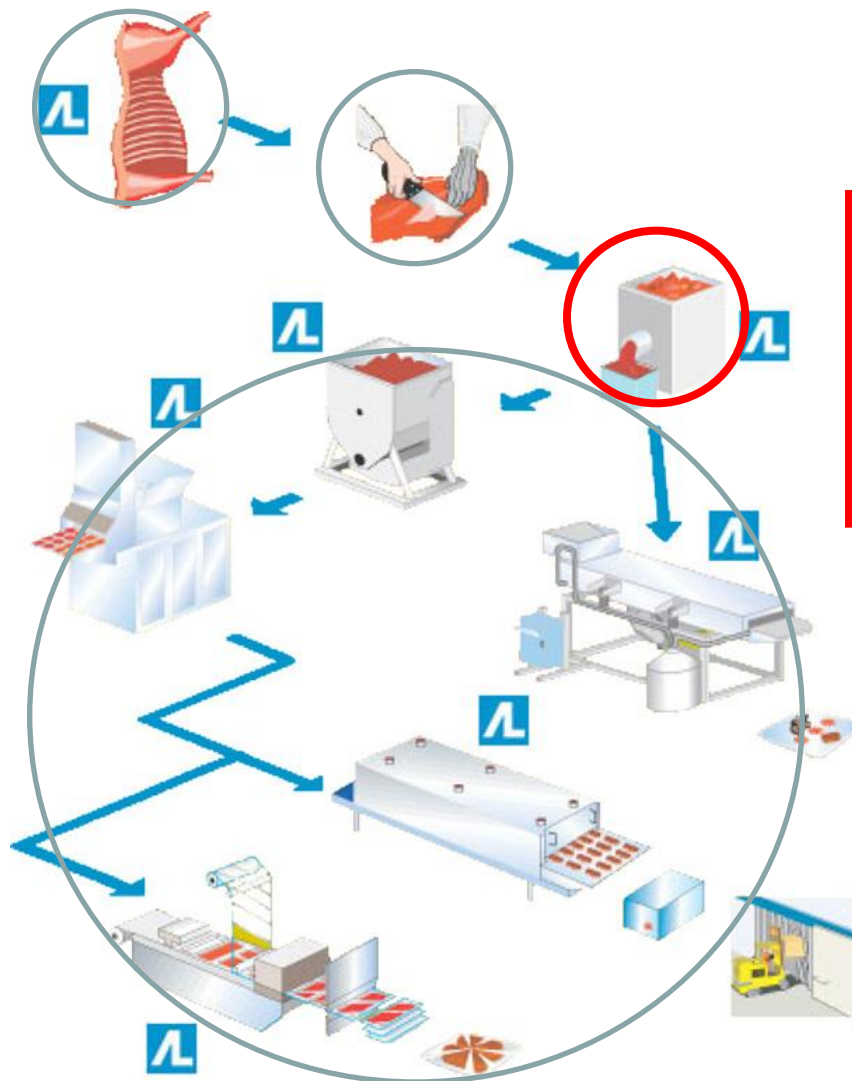
Background

- Horse and pork meat in processed beef products:
 1. How reliable are the methods for meat species and how low can they detect?
 2. Does cross contamination / carry over of meat species occur in UK meat processing plants when following GMP?
- Little or no information on this matter in the public domain
- FSA and Defra commissioned research

Project design - stakeholders

- Defra Authenticity Methods Working Group (Expert Group) – six meetings
- British Retail Consortium
- Food and Drink Federation
- British Meat Processors Association
- ABP Food Group
- Cranswick plc
- British Hospitality Association
- Leatherhead Food Research
- Association of Public Analysts
- Kosher and Halal Certifying Organisations

Risk based approach



Mincing:
Greatest risk when
no cleaning between
different red meat
species e.g. pork
followed by beef

Other risks – smaller

Project design - stakeholders

Aim:

To establish whether carry-over of meat species occurs when minced meat is produced according to good manufacturing practice in UK meat processing plants.

Species:

Raw pork in raw beef



- 1. Phase 1, Controlled study** - Determine the level of carry over that can be expected with, and without, equipment cleaning on change of species in a pilot plant.
- 2. Phase 2, Study of Industrial Practice** - Obtain information on the levels of carry over detectable in manufacturing plants with differing levels of control.

Risk categorisation

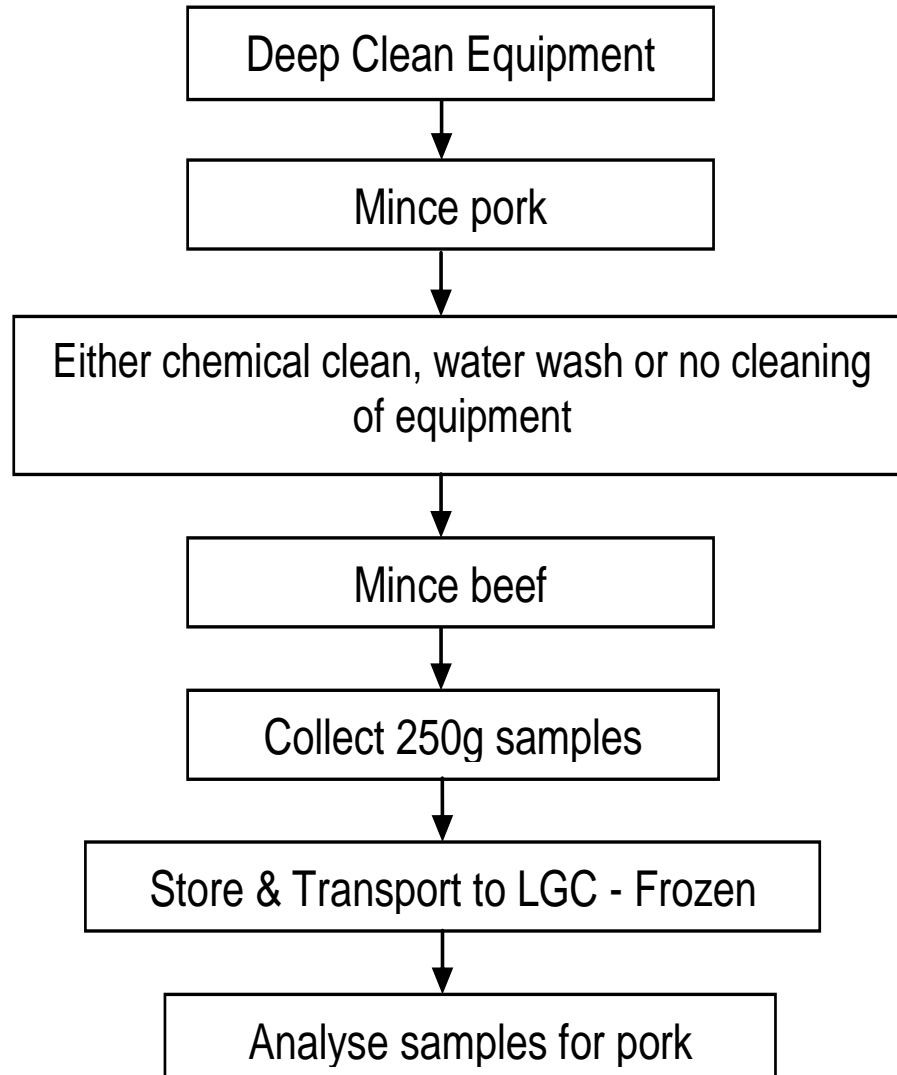
Handling of Meat Species

Categorisation	Risk Rating
Single species plant	Low
Multiple species plant that runs different species on different days on the same line	Medium
Multiple species plant that runs different species on the same day on the same line	High

Cleaning Regimes

Cleaning regime	Risk Rating
Chemical clean between species	Low
Equipment wash down with visual inspection between species	Medium
No wash down, flush through with different species	High

Process flow



Locking screw / ring (bottom left)



Validation

- LGC prepared QCs from authentic samples on w/w basis
- Commercial kits:
 - pork & beef – nuclear DNA
 - DNA Swabs - mitochondrial
- Validation required for meat samples & swabs
- Precision:
 - Repeatability
 - Intermediate precision
- Accuracy:
 - Pork content as % DNA
 - Pork content as % weight/weight
- Limit of Detection (LOD):
 - 0.1%
 - 0.03%

Acceptance criteria for QCs

QC Material	Expected Result
No template controls	No pork detected
100 % beef	No pork detected
Extraction blanks	No pork detected
Kit positive control	Corrected pork content: 70 – 130 %
Kit standards:	
PCR efficiency	85 -115 %
R2	>0.98
In-house w/w QCs:	
0.1 % pork in beef	Pork detected
50 % pork in beef	25 – 100 % pork
100 % beef	No pork detected



Phase 1 – Pilot Plant

Sampling plan

- Statistical input
- Authentic samples of pork and beef
- 100 kg of beef to be sampled per experiment
- Each 250g sample is thoroughly homogenised
- Each experiment is being replicated three times
- Single extraction & single PCR
- ~10% duplicates – repeatability
- 10 replicates of a high and low sample – repeatability varies with concentration?
- Swabs taken at critical places
 - Protein
 - ATP
 - DNA



Sampling plan



Cleaning	Overall Risk Rating	Experiment 1 - No. of samples of:			Experiment 2 - No. of samples of:			Experiment 3 - No. of samples of:			Total samples
		Input meat	After species change	Rest of batch	Input meat	After species change	Rest of batch	Input meat	After species change	Rest of batch	
Deep	Low	1	40	40	same	40	40	same	40	40	241
Environmental swabbing		10			10			10			30
Wash	Medium	1	40	40	same	40	40	same	40	40	241
Environmental swabbing		10			10			10			30
None	High	1	40	40	same	40	40	same	40	40	241
Environmental swabbing		10			10			10			30



Results – low risk

- No pork was detected in any sample to a LOD of 0.1%
- Protein swabs were all negative
- Pork DNA was not detected on any swabs
- ATP swabs were all deemed satisfactory

Conclusion:

‘Deep’ cleaning is effective in preventing the carry over of meat species

Results – medium risk

- No pork was detected in any sample to a LOD of 0.1%
- Protein swabs were all negative
- Pork DNA was not detected on any swabs
- ATP swabbing of some areas gave high results

Conclusion:

Cleaning using high pressure water is effective in preventing the carry over of meat species

Results – high risk

Pork content of beef samples post species change (~10kg)

Mincer

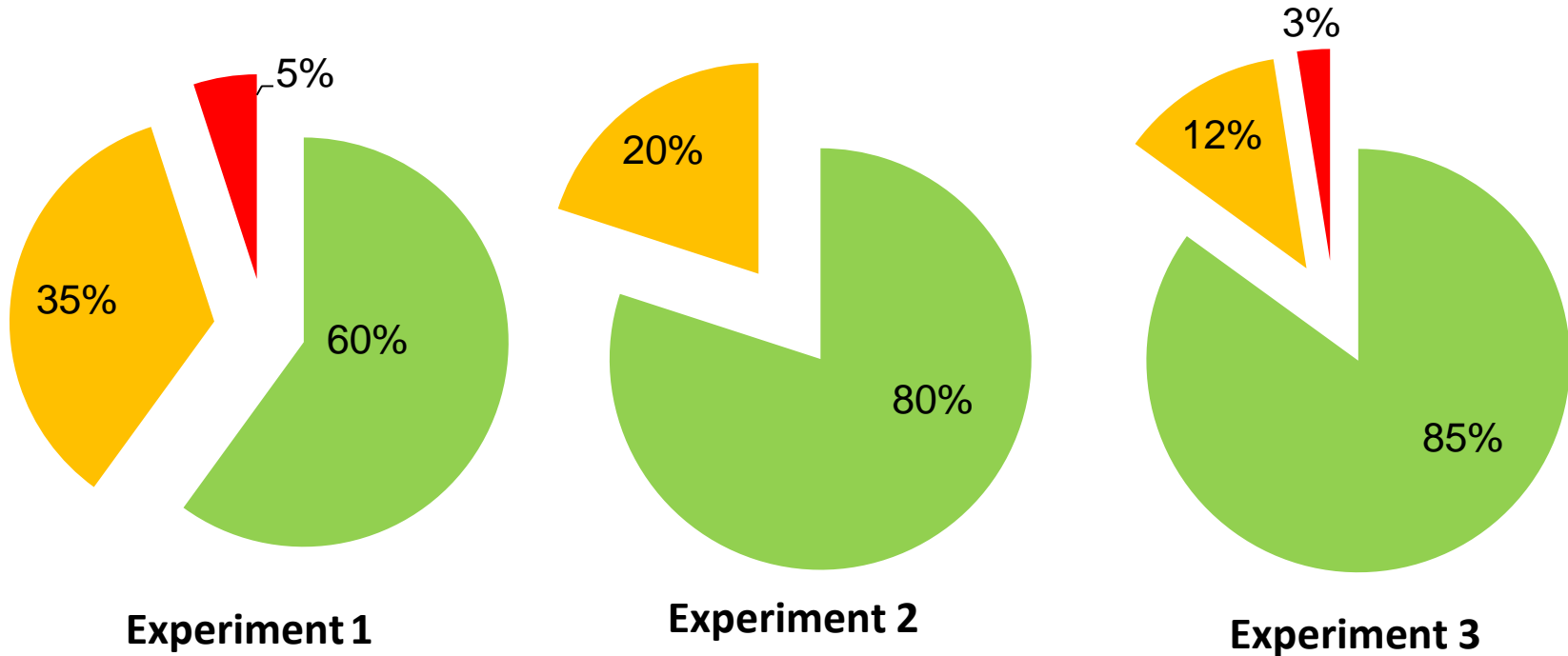
Meat
output



Sampling Plan			High risk – Experiment 1			High risk – Experiment 2			High risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	70.5	54.1	69.2	59.9	60.2	71.9	88.0	63.2	99.0
4	5	6	2.8	10.9	4.9	1.2	3.9	1.4	11.1	5.2	16.2
7	8	9	1.5	1.5	1.3	<0.1	3.7	0.9	5.6	3.2	0.7
10	11	12	0.9, 1.7	0.6	1.5	<0.1, <0.1	<0.1	<0.1	1.8, 1.7	8.0	1.1
13	14	15	0.8	3.9	1.2	<0.1	0.6	0.7	2.5	<0.1	0.2
16	17	18	0.5	0.9	0.6	1.2	0.5	0.9	1.0	<0.1	2.0
19	20	21	0.3	0.5, 0.9	0.4	0.4	0.8, 2.9	2.0	<0.1	0.5, <0.1	<0.1
22	23	24	<0.1	<0.1	0.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
25	26	27	<0.1	0.2	<0.1	<0.1	1.2	3.0	<0.1	<0.1	<0.1
28	29	30	0.3	<0.1	<0.1, <0.1	2.2	3.8	0.7, 1.2	<0.1	<0.1	<0.1, <0.1
31	32	33	<0.1	<0.1	<0.1	2.1	5.4	1.4	<0.1	<0.1	2.2
34	35	36	0.5	1.9	0.2	1.1	1.8	1.2	0.8	5.2	1.5
37	38	39	<0.1	0.4	<0.1	0.4	0.8	0.9	<0.1	<0.1	0.7
40	41	42	<0.1, 0.3			0.5, <0.1			<0.1, <0.1		

Phase 1 – high risk

Pork content of beef samples in remaining ~90kg



<0.1 %
>0.1 < 1 %
1-10%

<0.1 = LOD = pork DNA relative to mammalian DNA

Results – high risk

- Pork was detected in some samples
- Protein swabs were all negative
- Pork DNA was not detected on any swabs
- ATP swabs were all deemed satisfactory

Conclusion:

- When no cleaning is performed between species, carry over of pork meat into beef meat did occur
- Most significant in the first 0.75 kg of meat where concentrations ranged from 99% to 54% pork
- Total pork content of 100kg batch = 653g (0.65%)



Phase 2 – Manufacturing plants

Sampling plan

- 4 risk scenarios at 3 plants
 - Low: chemical clean
 - Medium: water wash
 - High: no clean
 - Single species plant*
- Typical batch sizes used
- Duplicate experiments (50 x 2)
- After species change
 - 3 samples taken directly
 - 18 samples taken consecutively and alternate samples analysed
- Remainder of batch
 - (100kg for chemical clean scenario and 200kg for water wash and no cleaning scenario)
 - 38 samples taken at increasing intervals
- Single analysis
- 10% duplicates



Sampling plan



Cleaning	Overall Risk Rating	Experiment 1 - No. of samples of:			Experiment 2 - No. of samples of:			Total samples taken	Total samples analysed*
		Input meat	After species change	Rest of batch	Input meat	After species change	Rest of batch		
Deep	High	1	21	38	-	21	38	119	101
Environmental swabbing		10			10			20	20
Water wash	Medium	1	21	38	-	21	38	119	101
Environmental swabbing		10			10			20	20
None	Low	1	21	38	-	21	38	119	101
Environmental swabbing		10			10			20	20



Results – low risk

- No pork was detected in any sample to a LOD of 0.1%
- Protein swabs were all negative
- Pork DNA was not detected on any swabs
- ATP swabs were all deemed satisfactory

Conclusion:

- ‘Deep’ cleaning is effective in preventing the carry over of meat species
- Confirmed the results from phase 1 – medium risk

Results – medium risk

- No pork was detected in any sample to a LOD of 0.1%
- Protein swabs were all negative except one
- Pork DNA was not detected on any swabs
- ATP swabbing of some areas gave high results
 - LOD for protein swabs stated by manufacturer as 50µg

Conclusion:

- Cleaning with high pressure water is effective in preventing the carry over of meat species
- Confirmed the results from phase 1 – medium risk

Results – high risk

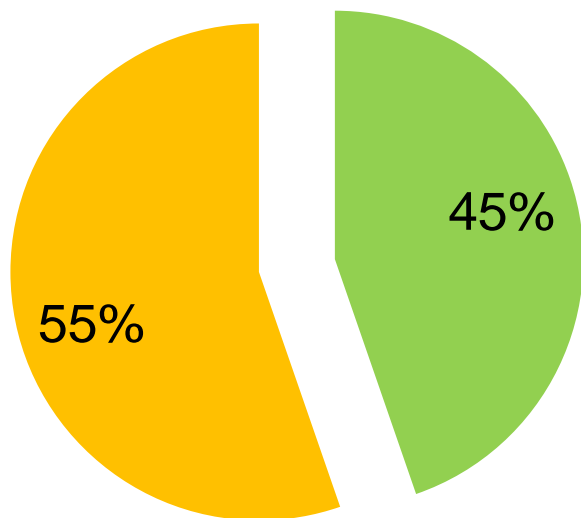
Pork Content of Beef Samples Post Species Change (~5kg)

		Experiment 1			Experiment 2		
Meat output ↓		97.2	97.8	94.7	184.4	162.9	145.2
		100.3		49.9, 46.2	144.7		98
			8.4			86.9	
		13.6		10.3	29.7		13.2
			5.8			6.2	
		2.7		5.7	4.8		2.9
			3.3			2.2	

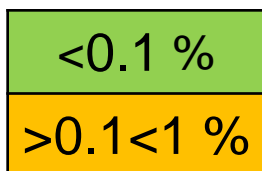
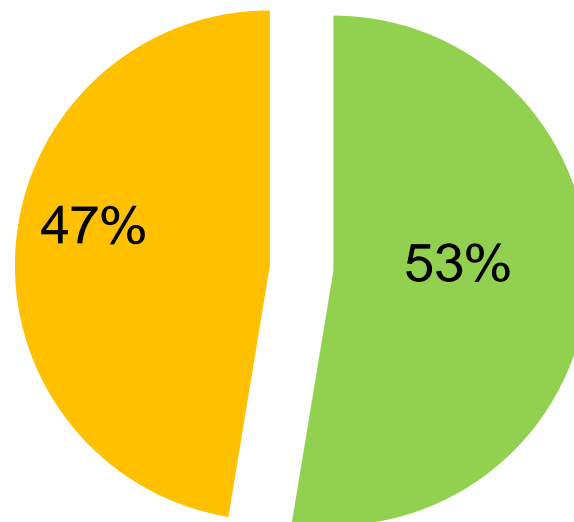
Phase 2 – high risk

Pork Content of Beef Samples in Remaining ~195kg

Experiment 1



Experiment 2



<0.1 = LOD = pork DNA relative to mammalian DNA

Results – high risk

- Pork was detected in some samples
- Protein swabs were all negative
- Pork DNA was not detected on any swabs
- ATP swabs were all deemed satisfactory

Conclusion:

- When no cleaning is performed between species, carry over of pork meat into beef meat did occur
- Most significant in the first 2.5 kg of meat where concentrations ranged from 100% to 30% pork
- Total pork content of 200kg batch = 11.2kg (5.6%)
- Confirmed the results from phase 1 – high risk

Results – Single species plant*

6 samples were taken at the beginning of each of two production batches

Experiments 1 and 2	
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1, <0.1



Interpretation of high risk scenario results

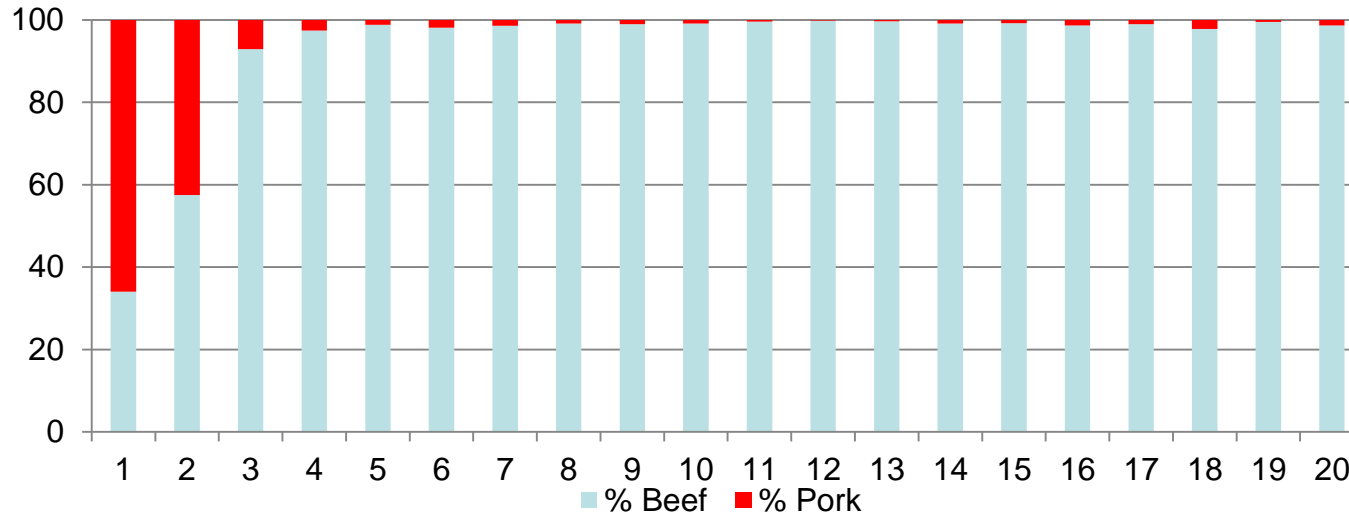
Summary

- Estimated pork content of 100kg beef processed in the pilot plant = 0.65kg (0.65%)
- Estimated pork content of 200kg beef processed in the commercial plant = 11.2kg (5.6%)
- For the pilot plant, no pork was detected after approximately 16kg, in the commercial setting, pork was detected in the last samples taken at 200kg
- Difference in results from phase 1 & 2 is likely due to differences in size and complexity of equipment

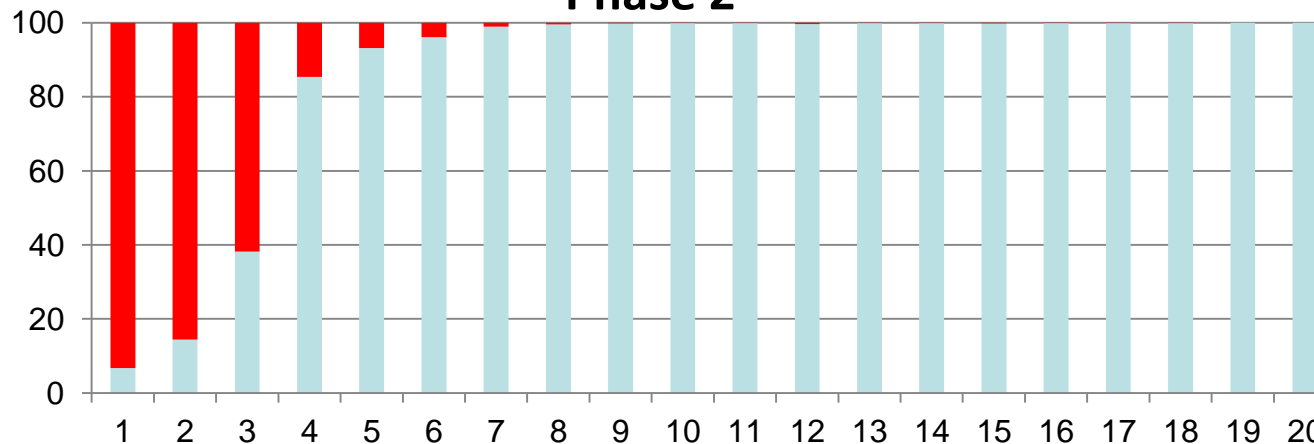
Pork carry-over into 500g retail packs of minced beef



Phase 1



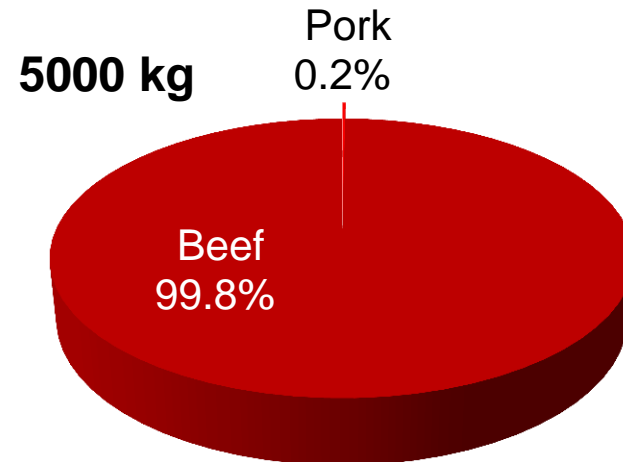
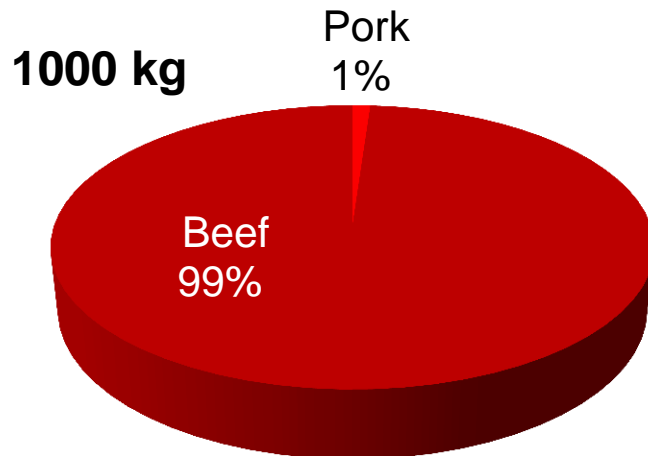
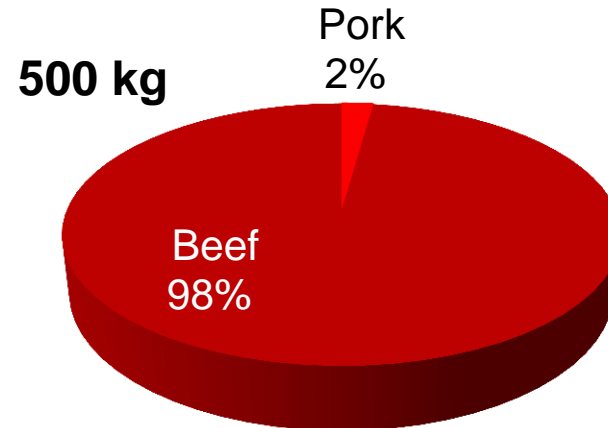
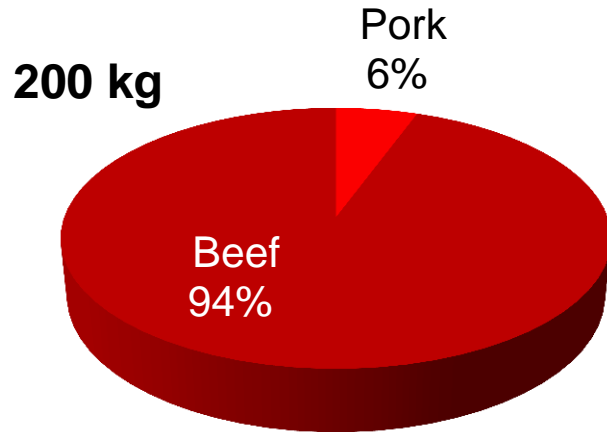
Phase 2



Mincer

Meat output →

Pork carry-over in beef burgers



Conclusions

- Carry over was not detected after deep cleaning or water washing
- When equipment wasn't cleaned, carry-over did occur:
 - 0.65% in 100kg – pilot plant
 - 5.6% in 200kg – manufacturing plant
 - Acceptable to consumers?
- Adventitious vs. deliberate
- Carry over can cause presence of undeclared species
- Swabs
 - Deep cleaning most effective
 - High ATP results were obtained after equipment was cleaned with a water wash
 - Pork DNA was not detected in any swabs
 - ATP swabs effective at checking efficiency of cleaning

Robustness vs limitations

- 1032 beef samples and 390 swab samples were analysed
- Robust – statistical, validation, stakeholder input
- Relevant – used UK industry practise
- Pilot & Manufacturing plants
- Carry over of raw pork into raw beef only was studied
- Only one commercial PCR kit was used for the determination of pork
- Only the mincing stage assessed
- Effect of different equipment was not investigated
- Carry over into real meat products not studied
- The LOD in processed products has not been studied

Final report

- [Defra](#) & [FSA](#) websites
- Publication in progress....

Acknowledgements

- Food Standards Agency
- Defra
- Analytical Methods Working Group (AMWG) and industry representatives for their valuable input throughout the project
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- LGC staff – Kirstin Gray, Joanna Topping, Rita Barallon & Malcolm Burns



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