Comparison of DNA extraction methods to detect *Salmonella* spp. from pig faeces and pork

Antonia Anna Lettini,
OIE, Italian Reference Laboratory for Salmonellosis

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Scientific background:

- ✓ *Salmonella* is an important food-borne pathogen
- ✓ Pork is recognised as one of the main source of human salmonellosis
- ✓ *Salmonella* Typhimurium (ST) is commonly associated with pigs

Thus……

- ✓ One needs to strictly monitor the presence of *Salmonella* at all stages from "farm to fork" along pig production chain
- ✓ An ever increasing interest is being devoted vs molecular methods which offer major sensitivity and specificity, furthermore, they are less laborious and time-consuming

….in particular

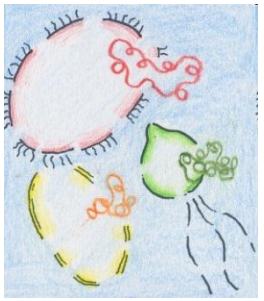
• Real-time PCR allows automation, computerization, and quantification of nucleic acids

yet:

• to obtain reliable results, several requirements are needed during sample preparation:

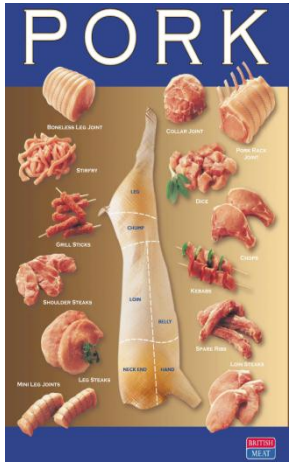
- (i) PCR-inhibitory substances must be removed,
- (ii) target nucleic acids or cells must be concentrated,
- (iii) heterogeneous samples must be converted into homogeneous samples
- (iv) detection of dead cells must be prevented

DNA extraction is quite SIMPLE and EASY to perform from pure bacterial cultures



BUT

DNA extraction from complex matrices along food chain



may result RATHER DIFFICULT

AIM

Evaluate different commercial kits for DNA extraction of *Salmonella* Typhimurium from pig faeces and pork

M&M

Pig faeces/pork samples:

- Sample preparation: 3 levels of ST/matrix, 3 samples/level
- Treatment or no treatment with Salmonella Dynalbeads
- DNA isolation by 4 different methods
- Measure of OD
- Real Time PCR analysis (commercial kit)
- Statistical analysis [by Linear Mixed Model]

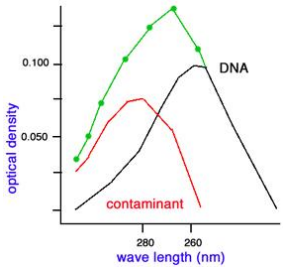
RESULTS

Comparison of DNA extraction methods by:

- DNA recovery (Quality and amount of DNA extracts)
- Analysis of Ct-value

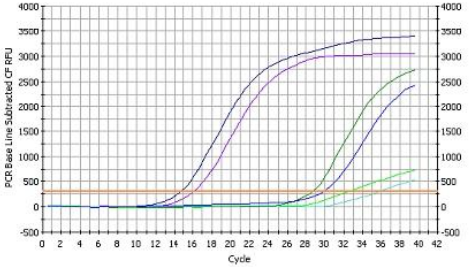
Pre-enrichment **Dynal-beads treatment** **DNA extraction** **DNA analysis**

Faeces



yes

no



Pork

Pig faeces:

M&M

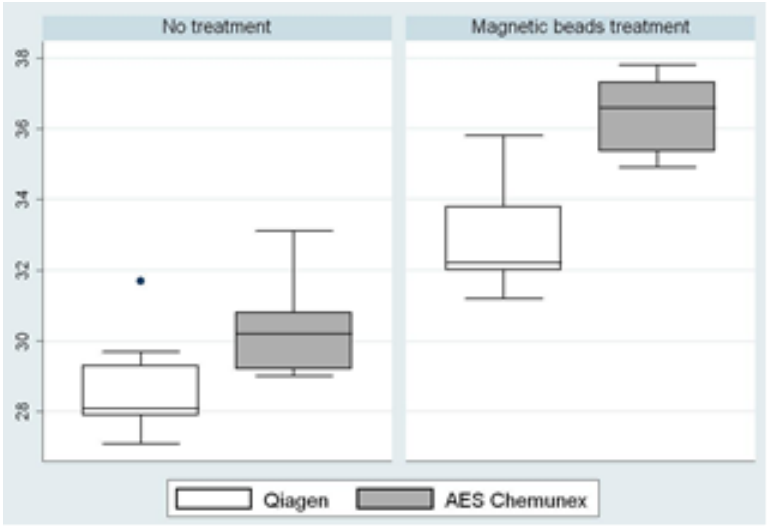
- Inoculum: 3 levels [24, 81, 136 CFU/10gr] and sample preparation
- DNA isolation: - Invitek
  - Qiagen
  - Lysis solution [Bio-Rad]
  - Extraction DNA mix [AES- Chemunex]
- Quality and DNA yield
- Real Time PCR analysis
- Statistical analysis

Initial inoculum CFU/10gr	Method	1		2		3		4	
	Name	InviMag Stool DNA		QIAmp DNA stool		Lysis reagent		Extraction DNA mix	
	Pretreatment	No	Yes	No	Yes	No	Yes	No	Yes
24 CFU/10gr		148	102	8	2,2	200	200	200	200
81 CFU/10gr		82	110	5	2,3	200	195	200	199
136 CFU/10gr		184	91	4	1,8	200	185	200	199

Pig faeces

Results

Recovery (μg/ml) of DNA from faecal samples spiked with ST (results are mean values determined for triplicate samples prepared at each concentration)



Box-and-whisker plots of distribution **Ct-values** from different extraction methods on faecal samples pretreated or not with Dynalbeads

Pork samples:

M&M

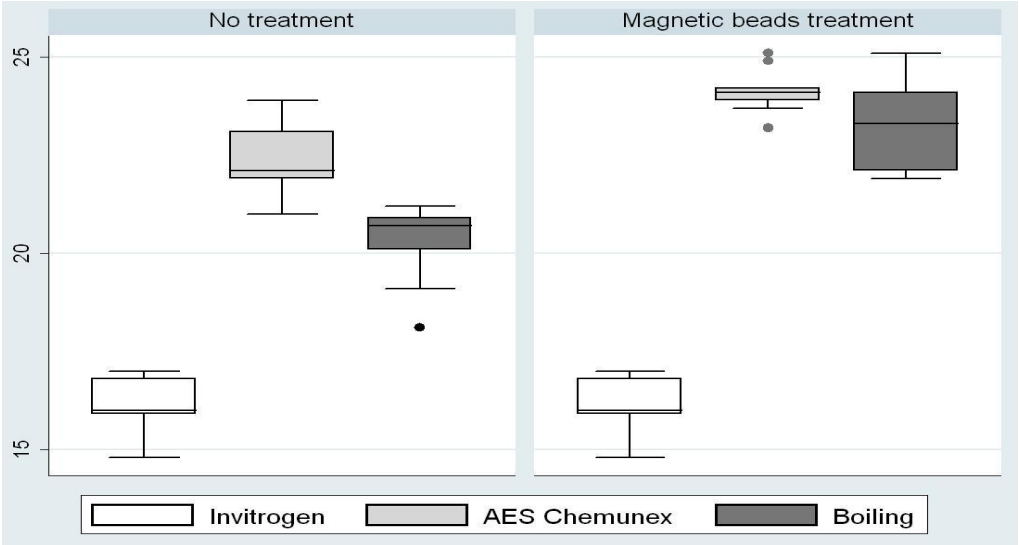
- Inoculum: 3 levels [40, 84, 119 CFU/10gr] and sample preparation
- DNA isolation: - Charge Switch gDNA mini bacteria
  - Lysis solution [Bio-Rad]
  - Extraction DNA mix [AES- Chemunex]
  - Boiling method
- Quality and yield of DNA extract
- Real Time PCR analysis
- Statistical analysis

Pork

Results

Initial inoculum CFU/10gr	*Method*	*1*		*2*		*3*		*4*	
	Name	Charge Switch gDNA mini bacteria DNA		Lysis reagent		Extraction DNA mix		Boiling method	
	Pretreatment	No	Yes	No	Yes	No	Yes	No	Yes
40 CFU/10gr		173	15	200	200	200	200	113	55
84 CFU/10gr		143	48	200	200	200	200	165	77
119 CFU/10gr		155	12	200	200	200	200	77	54

Recovery (μg/ml) of DNA from pork samples spiked with ST (results are mean values determined for triplicate saples prepared at each concentration



Box-and-whisker plots of distribution **Ct values** from different extraction methods on pork samples pre-treated or untreated with Dynalbeads

Conclusions

The efficacy of molecular methods, as Real Time PCR, can be enhanced by using **suitable** extraction methods for the matrix tested.
Thus, our results show that:

- among the different kits tested, 2 (for faeces) and 3 (for pork) are suitable to detect *Salmonella* by real time PCR kit
- ***boiling method***, possibly with some improvements, represents an inexpensive, handling and time-saving method to obtain *Salmonella* DNA;
- **pre-treatment** of samples with **Dynalbeads** does **not result in an improvement** of the DNA recovery and quality

Thank you for your attention