

Boar detection: industrial requirements and current state (EU)

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Topics

- Industrial method requirements of EU slaughter plants
- Current state detection methods at industry level in EU
- State of the art detection methods
- Missing up to date input

BoarCheck project 2013-2014 (SANCO/2012/SI2.639561)

State of the art of rapid detection methods used and in development

- Survey of methods
- Current industrial situation
- Identification and definition of industrial method specifications

Feasibility and cost assessment

- Definition of method performance requirements
- Critical review of method performance
- Cost assessment, implementation and development
- Integrated method evaluation (performance and cost)
- Priority list of selected methods

Workshop on rapid detection methods

Discussions and recommendations

Method comparison of prioritized methods

- Test of potential methods in development
- Test of methods currently in use at industry level

Industrial situation – questionnaires (BoarCheck)

Table 2.1: Number of responding slaughter companies, number of companies contacted prior to sending a questionnaire, number of <u>companies which</u> were sent a questionnaire, and the response rate per country.

Country	Number of	Number of companies	Response rate (%)
	respondents	a questionnaire was sent to	
EU			
Belgium	6	10	60.0
Denmark	2	3	66.7
France	8ª	10	80.0
Germany	2	5	40.0
Italy	0	7	0.0
Netherlands	3	5	60.0
Spain	8	23	34.8
United Kingdom	2	17	11.8
Third countries			
Canada	1	1	100.0
New Zealand	1	7	14.3
Norway	2	5	40.0
USA	0	1	0.0
Total	35	94	37.2

^a In France one respondent was not a slaughter company, but a company in charge of controlling carcass weight and carcass meat content. In this <u>report</u> we use the term 'slaughter companies' for convenience, but this also includes this company.

Industrial situation – slaughter line numbers EU

Table 2.3: Reported weekly number of slaughtered pigs, entire male pigs and immunocastrates and line speed of the EU slaughter companies, which responded to the questionnaire (q4, q5, q7, q9).

	Weekly number of slaughtered pigs				Line speed (pigs/hour)			
Animal type	Median	Min.	Max.	St.dev.	Mean	Min.	Max.	St.dev.
All pigs	20,000	800	400,000	106,456	428	40	800	173
Entire male pigs *	1,300	40	60,000	11,756	439	100	800	156
Immunocastrates ^b	575	400	750	247	580	560	600	20

Only those slaughter companies that slaughter entire male pigs.

b Only those slaughter companies that slaughter immunocastrates. This was not asked to the slaughter companies in Denmark, France and UK, because a draft version of the questionnaire was used.

100-800 entire males/hr: 36 – 4.5 sec pr carcass

Industrial situation – detection systems

Table 2.2: Number of slaughter companies, which responded to the questionnaire (q6^a, q8, q10), that reported to have a boar taint detection system and to slaughter or to expect to start slaughtering entire male pigs or immunocastrates in the next

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	_	_	-	_	

		Ν	lumber of resp	ondents						
	Expect to start									
	Slaug	hter	slaughtering	in next years	With boar taint					
	Entire male	Immuno-	Entire male	Immuno-	detection					
Country	pigs	castrates	pigs	castrates	system					
EU										
Belgium	5	3	0	2	2					
Denmark	2	n.a. ^b	0	n.a.	2					
France	3	n.a.	2	n.a.	4					
Germany	2	0	0	0	2					
Netherlands	3	0	0	0	2					
Spain	7	0	0	0	0					
United Kingdom	1	n.a.	0	n.a.	0					
Third countries										
Canada	0	0	0	1	0					
New Zealand	1	0	0	0	0					
Norway	0	1	1	0	0					
Total	24	4	3	3	12					

g6 = question 6 of the questionnaire (Annex1), of which this table provides the answers.

b This was not asked to the slaughter companies in Denmark, France and the UK, because a draft version of the questionnaire was used.

Industrial situation – detection systems

Table 2.4: General characteristics of the boar taint detection systems in use during spring 2013 as reported by the slaughter companies in the EU, which responded to the questionnaire (q17, q18, q20) and indicated to have a boar taint detection system.

		N	umber of re	spondents wit	h			
	Analysi	s method	Check of	entire males	Scale for I	Scale for boar taint		
Country	Sensory	Chemical	All	Sample	Yes/no	>2 levels		
Belgium	1	0	1	0	1	0		
Denmark	1	1	2	0	2	0		
France	3	0	3	0	2	1		
Germany	2	0	2	0	0	1		
Netherlands	2	0	2	0	1	1		
Spain	0	0	0	0	0	0		
United Kingdom	0	0	0	0	0	0		
Total	9	1	10	0	6	3		

Industrial situation – detection time and line speed

Table 2.5: Reported slaughter line speed and analysis time per carcass of the boar taint detection systems in use at slaughter companies, which responded to the questionnaire (q22, q23).

	Anal	ysis time per	carcass					
Method of boar		(seconds/pig	g)	Slaughter line speed (pigs/hour)				
taint analysis	Mean	Minimum	Maximum	Mean	Minimum	Maximum		
Sensory	5.2	2.0	8.0	511	350	680		
Instrumental	30.0	30.0	30.0	360	360	360		

For the sensory methods boar taint was defined on off-odour, androstenone and skatole smell and for the instrumental method on skatole concentration (Table 2.6).

Industrial situation – detection methods

Table 2.6: Basis for the definition of boar taint used in existing boar taint detection systems as reported by the slaughter companies, which responded to the questionnaire (q19).

Method of	Number	Number of respondents with boar taint definition based on								
boar taint	Androstenone	Skatole								
analysis	concentration	concentration	smell	smell	Off-odour					
Sensory	0	0	6	6	5					
Instrumental	0	1	0	0	0					

Table 2.7: Location of existing boar taint detection systems at the slaughter line as reported by the slaughter companies, which responded to the questionnaire (q24).

Number of respondents with location of boar taint detection system									
Method of boar	On-line after	On-line Just							
taint analysis	meat inspection	before cooling	Cooling area	Off-line					
Sensory	2	4	1	2					
Chemical	. 1	0	0	0					

Industrial situation – detection methods

Table 2.8: Sampling location, sample composition and sample analysis location of the existing boar taint detection systems as reported by the slaughter companies, which responded to the questionnaire (q25, q26, q27).

Method of		Number of respondents with sample characteristic								
boar taint	Samplin	g location	Sample composition	Sample anal	Sample analysis location					
analysis	Neck	Loin	Subcutaneous fat	On carcass	Elsewhere					
Sensory	8	1	9	6	3					
Instrumental	1	0	1	0	1					

Table 2.9: Sensitivity, specificity, reproducibility and repeatability of the existing boar taint detection systems as reported by the slaughter companies, which responded to the questionnaire (q28).

Method of boar taint analysis	Number of respondents that check accuracy	Mean % false negatives (min-max)	Mean % false positives (min-max)	Mean % reproducibility (min-max)	Mean % repeatability (min-max)
Sensory	6	11 (2-20)	21 (2-40)	98 (98-98)	98 (98-98)
Instrumental	1	n.p.ª	n.p.	n.p.	n.p.
^a Not provided			1	1	

* Not provided.

Industrial situation – detection methods

Table 2.17: Requirements for a boar taint detection system at the slaughter line as reported by the slaughter companies, which responded to the questionnaire (q11, q12, q13, q16).

		_						
	Boar taint detection system		Scale for boar taint		Reporting to farmer		Median maximum Costs (min. – max.)	
		Not	Yes /	>2			(€/tested entire	
Country	Needed	needed	no	levels	Yes	No	male pig)	
EU	26	5	26	2	19	5	0.50 (0.00-2.00)	
Third countries	1	3	3	0	3	0	0.04 (0.04-0.04) ^ª	

^a Data from one company.

Three companies provided initial investment costs of their sensory based boar taint detection system. Initial investment costs ranged from €3,600 to €12,000

Seven slaughter companies provided the running costs of their system. Six of them used a sensory method and one an instrumental method.

Median running costs for a sensory system were €1.50 per slaughtered entire male pig (range from €0.20 to €2.68) and €1.34 per slaughtered entire male pig for the instrumental system.

Industrial situation –

Table 2.19: Importance of specific aspects of a boar taint detection system at the slaughter line as reported by the slaughter companies, which responded to the questionnaire (q15).

^a 1 = not important, 5 = very important.

^b These questions were not asked to the slaughter companies in France.

		Fraction of respondents answering in each category				
Aspect of a boar taint detection system	1 ^a	2	3	4	5	
How easy it can be relocated physically	0.23	0.29	0.13	0.16	0.19	
How easy it can be adopted to changes in the slaughter line speed	0.13	0.10	0.13	0.23	0.40	
How easy it can be adopted to changes in boar taint parameters	0.09	0.00	0.04	0.17	0.70	
How easy it can be adopted to the number of entire male pigs slaughtered	0.14	0.00	0.17	0.17	0.52	
How easy it can be cleaned	0.07	0.07	0.03	0.30	0.53	
It does not conflict with food safety concerns	0.13	0.03	0.00	0.13	0.71	
How easy it can be technically maintained	0.10	0.00	0.10	0.27	0.53	
It must be fully automated	0.07	0.07	0.20	0.30	0.37	
It must be accepted by customers buying meat from entire male pigs	0.06	0.06	0.03	0.16	0.68	
It must not endanger labour conditions (work place safety)	0.00	0.04	0.07	0.07	0.82	
Measurement must be directly on the carcass in the slaughter line ^b	0.05	0.05	0.15	0.15	0.60	
Analysis result must be available immediately after the measurement ^b	0.05	0.00	0.10	0.29	0.57	

State of the art – rapid detection methods

<u>Method survey</u> (web search, patent search, consultancy and questionnaires - industry and res. groups)

Instrumental

- Spectrophotometry
- Gas-phase fingerprinting/Gas-sensor array
- Mass-spectrometry*
- Ion Molecule Reaction*
- Immunology*
- Biosensor*
- Gas chromatography*
- Liquid chromatography*

* Substance specific methods

Sensory

- Sensory perception with trained persons/Human nose

Method performance criteria - Instrumental methods

Method parameters	Requirements
Methods	1 method
Accuracy	i
Precision ⁱⁱ	≤ 10 %
Specificity	Free from matrix or spectral interferences
Limit of quantification (LOQ)	
LOQ in fat phase	0.05-0.10 μg/g S [™] , 0.10-0.25 μg/g A [™]
LOQ measurement on carcass/adipose tissue	0.025-0.05 μg/g S ⁱⁱⁱ , 0.05-0.12 μg/g A ⁱⁱⁱ
Method capacity	
Samples analysed pr hr	100-800 carcasses
Analysis speed per sample	4 – 40 sec
Sampling time per sample	<20 min
Result reporting	
Off-line method	< 30 min
On-line method	< 1 min
Costs	
Running cost per carcass	< 2.0 Euro

¹ agreement with the assigned value of a reference standard, or with the content derived by a reference method within maximum relative uncertainty of 10 %.. ^{ii,} The degree of agreement between independent measurements. The precision is set to 10 % with regard to the LOQ to account for a measurement uncertainty +/-10 % that assures correct classification of positive or negative sample at the sorting criteria levels. ⁱⁱⁱ Calculations for LOQ are based on the following suggested lowest limits for threshold levels : 0.20 µg/g for skatole and 1.0 µg/g for androstenone on fat basis.

Instrumental method's compliance to performance criteria

Method parameters	Require- ments	Spectroph	otometry	Gas- sensor array	Mass Spectrometry		Immuno- logical	Sensors	
		Colorimetric	FTIR		GC, LC	SIFT, PTR		Insects	Electrochem.
Methods	1 method	8	ОК	ОК	ОК	ОК	8	ОК	OK
Limit of quantification (LOQ)									
	Skatole	ОК	ND	ND	ОК	ND	8	ND	ОК
	Androsten.	8	ND	ND	ОК	ND	ОК	ND	8
Accuracy	(1)	ОК	ND	ND	ОК	ND	ОК	ND	ND
Specificity	(2)	ОК	ND	8	ОК	ND	ОК	ND	ND
Precision	<10 %	ОК	ND	ND	ОК	ND	ОК	ОК	ND
Method capacity									
Capacity/analysis pr hr	100-800	OK-🛞	ND	ND	ND	ND	ND	ND	ND
Analysis speed pr sample	4 – 40 sec	$\overline{\mathbf{i}}$	ND	ОК	$\overline{\otimes}$	ОК	\otimes	ОК	ОК
Sampling time pr sample	0.5-20 min.	ОК	ND	ND	ОК	ND	$\overline{\mathfrak{S}}$	Ok	ОК
Result reporting									
At-line method (3)	< 30 min	ОК	OK	ОК	ОК	ОК	$\overline{\mathfrak{S}}$	ОК	ОК
On-line method (4)	< 1 min	$\overline{\mathfrak{S}}$	\otimes	$\overline{\mathfrak{S}}$	$\overline{\mathfrak{S}}$	$\overline{\mathfrak{S}}$	8	ОК	ОК
Robustness		\otimes	ND	ND	ND	ND	ND	ND	ND
Maintenance		\otimes	ND	ND	ND	ND	ND	ND	ND
Costs (5)									
Running cost pr carcass	< 2.0 Euro	ОК	ND	ND	ND	ND	ND	ND	ND

ND : not documented or poorly documented ; 😕 : Not satisfactory compared to requirements

(1): Agreement with the assigned value of a reference standard, or with the content derived by

a reference method within max relative uncertainty of 10 %..

(2): Free from matrix or spectral interferences

(3): At-line: Sample is taken on the carcass and transferred to a laboratory where it is assessed by sniffers in controlled conditions.

(4): On-line: Sample is taken directly on the carcass and analyzed directly at the sorting band.

(5): Costs do not include chemicals, reagents and maintenance.

Method performance criteria Sensory/Human nose methods

Method parameters		Requirements
Methods		1 method
Accuracy		
	Sensitivity ⁱ	90-100 %
	Specificity ⁱⁱ	95-100 %
Precision		≤ 10 %
Method capacity		
	Capacity/analysis pr hr	100-800 carcasses
	Analysis speed pr sample	4 – 40 sec
	Sampling time pr sample	0.5-20 min.
Result reporting		
	Off-line method	< 30 min
	On-line method	< 1 min
Costs		
	Running cost pr carcass	< 2.0 Euro

¹% ability to identify positive samples as determined by a golden standard method. ^{II} % ability to identify negative samples as determined by a golden standard method.

Example: a 95 % sensitivity would mean that there will be 5 % false negatives Accordingly, the sensitivity should ideally be 100 % to avoid any false negatives

Sensory method's compliance to performance criteria

Method parameters	Require- ments	At-line (1) Hot water (3)	At-line Dry heating (4)	On-line (2) Dry heating
Methods	1 method	ОК	ОК	ОК
Accuracy				
Sensitivity	90-100 %	ND	☺ (6)	ND
Specificity	95-100 %	ND	☺ (6)	ND
Precision/reproducibility	<10 %	ND	ND	ND
Method capacity				
Capacity/analysis pr hr	100-800	$\overline{\otimes}$	ОК	ОК
Analysis speed pr sample	4 – 40 sec	$\overline{\mathfrak{S}}$	ОК	ОК
Sampling time per sample	0.5-20 min.	$\overline{\otimes}$	ОК	ОК
Result reporting				
At-line method	< 30 min	ОК	ОК	-
On-line method	< 1 min	-	-	ОК
Robustness and maintenance		The key issue is the quality control of the sniffers		
Costs (5)				
Running cost per carcass	< 2.0 Euro	ОК	ОК	ОК

ND : not documented or poorly documented

 $\boldsymbol{\boldsymbol{\otimes}}$: Not satisfactory compared to requirements

(1): At-line: Sample is taken on the carcass and transferred to a laboratory where it is assessed by sniffers in controlled conditions.

(2): On-line: Tissue is heated and sniffed directly on the carcass.

(3): Hot water: the sample is soaked for 2 minutes in a flask containing hot water and sniffed at the opening of the flask (Meinert et al., 2011)

(4): Dry heating: the sample (or the tissue on the carcass) is heated for a few seconds (soldering iron, hot air, ...) and sniffed immediately.

Recommendations instrumental methods

- The study has shown that there are a few **instrumental methods** under development which can potentially be applied for on-line detection of boar taint. However, further research on the development and full validation of these methods under industrial conditions is required before they can be considered for application in an industrial setting.
- To perform an evaluation of the accuracy and sensitivity of the instrumental methods under development, as described in BoarCheck WP4. However, sufficient resources should be provided in order to enable such evaluation against currently used chemical analysis of skatole and androstenone as well as sensory analysis.
- Instrumental analysis should be compared to sensory analysis such as conclusions on the agreement between both types of quality control can be drawn.
- Since boar taint acceptance levels may vary between countries, product types and customers, that would require different threshold levels of androstenone and skatole for sorting purpose, instrumental methods should be able to handle various threshold levels.

Recommendations sensory methods/Human nose

- The accuracy of human nose methods should be evaluated at industry level. The proposal in BoarCheck WP4 describes a protocol to achieve that, using AIS levels as gold standard for boar taint, and in addition sensory assessment with a trained expert panel. However, sufficient resources should be provided in order to enable such evaluation against currently used chemical analysis of skatole and androstenone as well as sensory analysis.
- The performance of the human nose methodology is poorly documented. We recommend that the results of the human nose methods are validated for performance as suggested in the method comparison study report (D4.1) in order to determine their sensitivity and specificity under real industrial conditions. This would also require sufficient resources allocation.
- The performance of the on-line and at-line methods should be compared to evaluate the effect of the on-line conditions on the sensory evaluation of boar taint.
- Method performance of the experts performing the human nose methodology should also be compared to the method performance results of trained expert panels on-line as well as at-line, both in relation to the results of the chemical analysis in order to set achievable criteria for the human nose methodology.
- The method comparison protocols prepared for evaluating the human nose methodology and evaluating the selection and training procedure of the experts performing the human nose methodology (WP4) may be useful for further research in this field. However, sufficient resources should be provided in order to enable evaluation of method performance compared to chemical analysis as well as sensory analysis.

Recommendations for a boar taint reference standard

- A verifiable reference standard for boar taint is extremely critical, both for the development of detection methods and the development of strategies to reduce boar taint.
- To account for the different views on a reference gold standard, a discussion with academic and industry scientists should take place, to envisage possible ways of going around the problem of the missing universally recognised gold standard for boar taint.
- There are a number of reasons why **this issue should be addressed collectively**, in an international project funded by the involved stakeholders:
 - The research to address this issue is **generic**, not competitive.
 - The research to be conducted involves large scale consumer acceptance studies, which are extremely costly.
 - The research must be conducted at an international level as it is important to take into account variability and diversity, in eating culture, perception of boar taint, and human inter-individual variability of olfactory perception. The same methodological approach should be used in international studies to achieve reliable results.

Industrial situation – EU (state 2014....)

- Boar taint detection is an issue in EU countries
- Entire male pigs are slaughtered in the EU (B, DK, F, NL, G, ES, UK)
- 40-60.000 slaughtered pr week (1300 median)
- Four companies (in Belgium and Norway) indicated to slaughter immunocastrates, and two companies expect to start slaughtering them in the next years
- Two companies (in Spain and Norway) indicated that they did not slaughter nor expected to start slaughtering entire male pigs or immunocastrates in the next years
- Boar tainted meat is sold to specific markets or used in meat products consumed without heating or mixed into non-tainted meat products
- Detection methods are being used at major slaughter plants at industry level (B, DK, F, NL, G)
- Sensory based methods (human nose) used as routine sorting method and one instrumental method (DK)
- Various protocols are being used for the sensory methods (sample location, heating method, training of assessors etc.)
- Instrumental methods (both Skatole and Androstenone) still needed

State of the art detection methods

- Update required on potential detection methods
- And methods in development
- •

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https://ec.europa.eu/food/sites/food/files/animals/docs/aw_prac_farm_pigs_cast-alt_research_boarcheck_20140901.pdf