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# On-farm prevalence of and potential risk factors for boar taint

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### ABSTRACT

Boar taint is an unpleasant taste and odor that can occur in entire male pigs and is caused by androstenone, skatole, and to a lesser extent indole accumulating in fat tissue. In the present observational study, we evaluated an extensive list of such potential risk factors which influence boar taint: social hierarchy and puberty attainment, housing, health, preslaughter conditions, season, feed, carcass composition, slaughter weight or age, and breed. Details on these factors were collected by interviews with the participating farmers, observations on each farm by trained observers and farmers, as well as slaughterhouse data. Twenty-two farms (in West- and East-Flanders, ranging from 160 to 600 sows, selected on suitability) raising entire male pigs were included in the study to evaluate the link between boar taint and potential risk factors related to the farm and slaughter batch (114 slaughter batches and 16 791 entire male pigs in total). Average olfactory boar taint prevalence was 1.8  $\pm$  0.8%. Boar taint prevalence varied also within farms up to a maximum range between slaughter batches of 9.1% which suggests an effect of factors varying between slaughter batches such as season or other variables varying between slaughter batches. Less aggressive behavior at the end of fattening as well as lower skin lesion scores at fattening as well as at slaughter could be associated with less boar taint. The same might be said for sexual behavior, though less convincingly from this study. Measures that reduce aggression and stress have therefore have the potential to lower boar taint prevalence. The same might be said for sexual behavior, though less convincingly from this study. Furthermore, boar taint prevalence was generally higher in winter than in summer, which is relevant from a planning perspective for the slaughterhouses to seek alternative markets. Finally, increased CP gave significantly lower boar taint prevalences. This may to some extent be explained by the negative association between boar taint and lean meat percentage, as increased dietary CP levels promote the carcass lean meat percentages which can then be associated with lower boar taint levels.

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#### Implications

Measures that reduce aggression and stress on the farm in the time pre-slaughter likely reduce boar taint prevalence. Boar taint prevalence was higher in winter than in summer, this can be taken into account by slaughterhouses or wholesalers for marketing purposes. Crude protein is negatively linked with boar taint but needs further research to find the causal relation. Breeding for higher lean meat percentage may lower boat taint.

### Introduction

The European pig sector has made a commitment to stopping surgical castration of male piglets by 2018, even though this deadline has

Corresponding author. E-mail address: Marijke.aluwe@ilvo.vlaanderen.be (M. Aluwé). passed, efforts continue to be made to make this transition possible (Bonneau and Weiler, 2019). Raising uncastrated or entire male pigs is one possible alternative but has an associated risk of boar taint occurring in about 5-10% of the pig carcasses (Aluwé et al., 2014; Backus et al., 2016; Channon et al., 2018; Bonneau and Weiler, 2019). Boar taint is an unpleasant taste and odor that is caused by the accumulation in fat tissue of androstenone (AND), skatole (SKA), and to a lesser extent indole (IND). In entire males, AND is produced in the testes while SKA and IND are produced by micro-organisms in the gut, AND further inhibits SKA catabolism in the liver leading to higher deposition (Rius and García-Regueiro, 2001). The olfactory evaluation of boar taint or the human nose method is mostly used to evaluate boar taint and is currently the Europe an Union reference for boar taint evaluation in practice. It has to be kept in mind that this remains a subjective method with limited accuracy, furthermore, to reduce assessor fatigue interstimulus intervals are required (Mathur et al., 2012; Trautmann et al., 2014).

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In the present study, we evaluated an extensive list of potential risk factors. Each of these potential risk factors which can be related to boar taint: social hierarchy and puberty attainment, housing, health, pre-slaughter conditions, season, feed, carcass composition, slaughter weight or age, breed (Fig. 1). Aggressive and mounting behavior (Giersing et al., 2000) and skin lesions (Bekaert et al., 2012a) have been linked with higher AND concentrations as these factors are related with social hierarchy and puberty attainment. For housing, a lower number of pigs per pen (van Wagenberg et al., 2013), the presence of gilts (Fàbrega et al., 2011), soiling and stocking rate (Hansen et al., 1994), floor type (Maw et al., 2001), and ventilation type (Hansen et al., 1994) have been linked with boar taint. Also dysentery and antibiotics have been linked with boar taint (Škrlep et al., 2012). A multitude of feed-related characteristics have been shown to affect boar taint levels (Zamaratskaia and Squires, 2009; Urbanová et al., 2016). The effect of lean meat percentage (Walstra et al., 1999) has been found in intervention as well as observational studies and could be linked to genetics (Dugué et al., 2020). The effect of carcass/live weight has been found in intervention studies suggesting an effect of age or maturity on boar taint prevalence (Walstra et al., 1999). Finally, also breed and genotype effects on boar taint have also been reported (Zamaratskaia and Squires, 2009; Zadinová et al., 2016).

The goal of this study was to increase the insights in boar taint prevalence and its variation between and within farms and to identify and assess the importance of risk factors for olfactory boar taint from literature in practice.

# Material and methods

#### Farms, animals, and neck fat sampling

Twenty-two farms raising entire male pigs were included in the study to evaluate the link between boar taint and potential risk factors related to the farm and slaughter batch. All farms were located in West- and East-Flanders and were selected on availability (farms producing entire male pigs), willingness to cooperate, and sufficient number of entire male pigs per slaughter batch and delivery or pigs to the collaborating slaughter houses. The study was conducted from September 2014 until January 2016. Information on these potential risk factors was collected based on questionnaires taken on location, the farmers logbook, animal observations, and slaughter batch-related data. On all farms, on average four groups of entire male pigs per farm were included in the study, with an average 200 pigs per group. The time periods between these groups were an average 60 days (minimum 10 to maximum 394 days). Each group was slaughtered in one or up to four slaughter batches, resulting in 114 slaughter batches in total or five slaughter batches per farm on average. Average number of entire male pigs per slaughter batch was 148. In total, 16 791 neckfat samples (cut from each carcass measuring  $5 \times 5 \times 5$  cm, corresponding to the total number of entire male pigs) were collected in the slaughterhouse and transported to the lab for sensory analysis of boar taint (method detailed below).



Fig. 1. Schematic summary of potential risk factors collected and their observational level (farm, slaughter batch, or animal). The aspect of boar taint risk to which each potential risk factor belongs is indicated on the right.

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## Farm and group-related questionnaires

Each farmer filled out a questionnaire during an interview at the start of the study and per group a smaller questionnaire was taken to collect group specific data to capture if anything in the management had changed between groups. When more convenient or to double check answers given, a set of potential risk factors were recorded by a trained assessor present in the compartment per group (indicated with '(M)' in Tables 1 and 2). These were mainly highly variable factors such as housing characteristics and group composition which could be easily registered during the animal observations.

Weekly observations of behavior, soiling, and health status by the farmer

The farmer was given a logbook for weekly monitoring of each group to follow-up health status behavior and soiling. Health status (healthy,

#### Table 1

Summary of continuous potential risk factors evaluated on 22 farms raising entire male pigs and their relation to boar taint prevalence (**BT** (%)) as determined by a trained olfactory panel. This is presented as the prevalence of the pigs with the lowest 25% parameter values (1st quantile) and of the highest 75% parameter values (3rd quantile). A mixed binomial model was used to determine significant relations between each potential risk factor and olfactory boar taint. The number of samples in each analysis is given by *n*.

	Mean	SD	Min.	Max.	BT (%) for 1st quantile	BT (%) for 3rd quantile	n	P-value
Social hierarchy & puberty Behavior (M)								
Inactive (%)	86.70	5.37	64.01	97.79	1.90	2.02	15 626	0.950
Eating & drinking (%)	4.42	2.99	0.00	11.91	1.89	1.69	15 626	0.986
Moving (%)	0.44	0.30	0.00	1.39	1.59	2.36	15 626	0.452
Interaction (%)	1.33	0.80	0.00	5.86	1.37	2.25	15 626	0.397
Manipulation (%)	6.04	3.04	0.84	15.87	2.01	1.84	15 626	0.811
Playing (%)	0.02	0.08	0.00	0.53	1.85	1.89	15 626	0.541
Ear & tail biting (%)	0.06	0.08	0.00	0.28	1.70	2.32	14 507	0.265
Aggression (%)	0.24	0.25	0.00	1.26	1.60	2.62	15 626	< 0.001 ***
Mounting (%)	0.35	0.36	0.00	2.02	1.47	1.95	15 626	0.464
Ano-genital sniffing (%)	0.35	0.28	0.00	1.73	1.43	2.37	15 626	0.092 °
Week of estimated puberty attainment	23.36	3.95	14.00	32.00	2.51	1.35	5 308	0.120
Percentage of pens with gilts (%) (M)	2.00	3.92	0.00	14.29	1.74	1.85	14 026	0.063°
Skin lesion in compartment (M)	1.14	0.39	0.31	2.08	1.21	2.57	15 417	0.002 **
Housing								
Length of time the pens are empty in between age groups (days)	7.74	3.07	3.00	14.00	1.74	1.92	13 832	0.746
Animal soiling (M)	1.21	0.34	0.80	2.94	1.78	1.42	15 626	0.245
Pen soiling (M)	0.32	0.36	0.00	1.67	2.22	1.48	15 626	0.105
Average soiling final 2 weeks	1.92	0.88	0.00	3.00	1.71	2.33	7 494	0.260
Surface area per pig (m <sup>2</sup> ) (M)	0.78	0.08	0.63	1.25	2.32	1.91	14 238	0.885
Lux (M)	27.30	13.36	6.80	69.50	1.70	1.53	10 389	0.828
Slat width (cm) (M)	8.71	2.31	5.50	18.20	2.29	1.53	14 379	0.033 *
Slot width (cm) (M)	1.77	0.25	1.20	2.40	1.91	1.35	14 379	0.288
Slot/slat ratio (M) (%)	17.37	2.82	9.90	25.26	1.55	1.85	14 379	0.182
Pen surface area (m <sup>2</sup> ) (M)	9.82	1.48	6.60	12.28	2.12	2.03	14 238	0.584
Stocking rate (M)	12.62	1.58	8.00	16.50	1.92	1.80	15 626	0.864
Health								
Mortality (%)	2.77	1.62	1.00	7.00	1.98	1.33	7 232	0.018 *
Pre-slaughter								
Loading duration (min)	75.32	26.90	30.00	140.0	2.06	1.75	7 421	0.391
Transport duration (min)	63.57	51.39	2.50	245.0	1.63	2.01	14 497	0.242
Pre-unloading duration (min)	5.95	2.25	0.00	10.00	2.24	1.32	12 936	0.633
Unloading duration (min)	16.64	4.69	10.00	30.00	2.53	1.89	8 /86	0.809
Duration in lairage (min)	95.09	72.10	7.00	330.0	2.59	1.34	14 840	0.010*
Fasting period (hours)	22.86	8.21	12.00	45.00	1./5	2.23	11466	0.098
Skin lesion score after slaughter (M) Season	0.81	0.64	0.00	3.00	1.87	1.96	7 422	< 0.001
Average outside temperature (°C)	7.49	4.81	-3.30	19.70	3.12	1.82	8 290	0.098°
Day length (h)	11.16	2.54	7.93	16.52	1.90	1.42	16 791	0.119
Feed	10.11	2.40	2.02	45.00	0.44	1.00	10.040	0.454
Pigs per feeding place (M)	10.11	3.19	3.83	15.00	2.11	1.23	12 343	0.454
Pigs per drink nipple (M)	6.10	2.44	2.48	12.92	2.03	1.64	12 588	0.167
Final phase feed composition	1451	0.55	12.20	15.00	2.25	0.17	11 110	0.020*
Crude protein	14.51	0.55	13.20	15.60	2.35	2.17	11 110	0.028
Crude Ial	4.52	0.08	2.97	5.40	2.01	1.38	11 110	0.450
Crude dsll	4.75	0.30	4.12	5.62	2.31	2.21	11 110	0.747
Lucina content	4.69	0.50	3.50	5.81 1.02	2.13	2.23	10 656	0.214
Lysine content Average daily gain (lyg/day)	0.92	0.00	0.80	1.05	1.79	1.19	10 000	0.150
Carcass composition	0.40	0.08	0.00	0.71	2.20	2.17	12710	0.018
Loop most percentage (%)	65.00	2 75	50.60	76.00	2 79	1.67	14 256	~0.001***
Age or weight	03.09	2.13	50.00	70.00	2.10	1.07	14 200	< 0.001
Carcass weight (kg)	94 69	11.66	52.00	141 40	2.02	2.02	15 011	0.876
Weaning age (days)	22.68	4 1 8	13.00	29.00	2.02	1 91	13 764	0.594
Fattening age (days)	74 94	10.45	59.00	103.00	1.81	1.72	13 561	0.164
Slaughter age (days)	204.04	13.69	171.00	239.00	2.24	1.97	14 006	0.555

Significance is indicated by: °0.1; \*0.05; \*\*0.01; \*\*\*0.001.

Potential risk factors evaluated by a trained assessor present in the compartment per age group are indicated with '(M)'.

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#### Table 2

Summary of factorial potential risk factors collected on 22 farms raising entire male pigs and their relation to boar taint prevalence (BT (%)) as determined by a trained olfactory panel. This is presented as the prevalence of olfactory boar taint (OLF) and the mean values for the boar taint compounds per factor level. A mixed binomial model was used to determine significant relations between each potential risk factor and olfactory boar taint. The number of samples in each analysis is given by *n*. The percentage of samples per factor level included in the olfactory boar taint analysis is given by *n*/level (%).

	n/category (% animals)	n (OLF)	BT (%)	P-value
Social hierarchy & puberty				0.1.10
Presence of gilts in compartment (M)	33.0	4 891	1 55	0.146
Yes	67.0	9 924	2.08	
Housing				
Cleaning method				0.485
No	20.0	2 769	2.35	
Wet	7.5	1035	1.04	
Distraction material present in the	1210	10 020	1102	0.111
pen (M)				
Chains	91.2	14 247	1.94	
Chains +	8.8	1 379	1.31	0.271
Natural light	22.0	3 432	1 78	0.371
Indirect natural light	22.4	3 496	1.60	
Direct natural light	45.6	8 698	2.05	
Floor type (M)				0.239
Curved (partly)	27.8	4 339	1.68	
FIGE Pen separation type: number of open	12.2	11 287	1.97	0.005**
sides (M)				0.005
0	15.5	2 139	2.66 <sup>ab</sup>	
1	13.4	1 845	2.17 <sup>ab</sup>	
2	62.4	8 616	1.53 <sup>a</sup>	
3	6.1	841	2.50 <sup>ab</sup>	
4 Ventilation system (M)	2.0	300	4.23	0.487
Natural	21.4	3 339	1.86	0.407
Door	9.8	1 538	1.56	
Underground air channel	41.2	6 445	2.19	
Perforated ceiling	8.8	1 374	1.82	
Air inlet valves	18.8	2 930	1.47	
Disease				0 234
Healthy	55.9	4 122	2.04	0.234
Diarrhea	12.6	930	2.90	
Coughing	31.5	2 318	1.98	
Antibiotics used				0.252
No	53.6	4 063	2.31	
Yes Anthelmintic	46.4	3 5 1 3	1.85	0.714
No	86.7	6 572	2.11	0.714
Yes	13.3	1 004	1.99	
Pre-slaughter				
Delivery strategy				0.627
All-in-all-out	58.3	7776	1.99	
Spiit marketing First or later delivery	41.7	2 221	1.95	0 332
First delivery	80.0	13 430	1.85	0.552
Later delivery	20.0	3 361	2.11	
Presence of gilts during transport				0.601
No	33.3	4 570	1.90	
Yes	66.7	9 1 4 2	2.02	
Season				0.005
Winter (DJF)	26.0	4 374	2.26 <sup>b</sup>	0.005
Spring (MAM)	10.7	1 798	2.46 <sup>b</sup>	
Summer (JJA)	16.1	2 703	1.42 <sup>ab</sup>	
Autumn (SON)	47.1	7 916	1.42 <sup>a</sup>	
Feed				0.701
Pellet	71	1 094	2 38	0.701
Mash	69.0	10 701	1.85	
Slurry	23.9	3 705	1.89	
Type of feeder (M)				0.876
Dry feeder	30.1	4 612	1.91	

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#### Table 2 (continued)

	n/category (% animals)	n (OLF)	BT (%)	P-value
Wet-dry feeder	45.7	7 000	1.90	
Liquid feeding	24.2	3 705	1.89	
Feeding system (M)				0.835
Liquid feeding	24.4	3 705	1.89	
Mash in dry feeder	22.3	3 392	1.80	
Mash in wet-dry feeder	46.1	7 000	1.90	
Pellets provided in dry feeder	7.2	1 094	2.38	
Feeding strategy				0.662
Restricted	15.9	2 050	2.10	
Ad libitum	84.1	10 870	1.98	
Number of feed phases				0.729
2	14.4	1 831	1.86	
3	26.6	3 391	1.95	
4	48.0	6 120	1.93	
5	11.0	1 402	2.50	
Breed				
Type of sow line				0.421
Danbred	42.7	5 911	1.83	
Hypor	10.1	1 398	2.15	
Large white	12.1	1 670	1.44	
PIC (Pig Improvement Company)	4.2	582	2.92	
Rattlerow Seghers	4.8	667	2.10	
Topigs 20	26.1	3 604	2.28	
Type of sire line				0.588
Pietrain selected for high daily gain	6.1	606	2.07	
Pietrain selected for high carcass	80.1	8 062	2.64	
quality				
Pietrain selected for high daily gain and carcass quality	13.1	1 307	1.53	

<sup>a,b</sup>Significant differences are indicated by different superscripts for alpha = 0.05. Potential risk factors evaluated by a trained assessor present in the compartment per age group are indicated with '(M)'.

diarrhea, coughing), actions taken (antibiotics: no/yes, anthelmintics: no/yes), and death cases were recorded. Next to this, the farmer scored aggressive and sexual behavior on a scale from 0 (never) to four (most of the time) per group. From these scores, the approximate week of puberty attainment was estimated (viewed as a rise in the relative score, not based on absolute score as this could vary from one farmer to the next). Average pen soiling was also scored on a scale from 0 (clean) to four (heavy soiled). The average soiling score for the last two observations before slaughter was further used for statistical analysis to evaluate the effect of soiling on boar taint prevalence.

# Evaluation of skin lesions, soiling, and behavior at the end of fattening by a trained assessor

One week before the first slaughter batch of a group, observations were performed on the farm by one single trained assessor for that entire group based on protocols used in previous studies (Bekaert et al., 2012b). Observations were always performed in the afternoon as pigs are generally more active then (Ingram and Dauncey, 1985).

Per group, each pen (on average 16 pens with 13 animals per pen) was scored by the trained assessor by passing along the pens and recording the number of pigs in each category per pen for live skin lesions and animal soiling, and the entire pen was assigned to a specific category for pen soiling. Skin lesions were scored from 0 (no scratches) to five (multiple severe wounds), animal soiling was scored from 0 (pig is clean) to four ( $\geq$ 75% of the pig is soiled), and pen soiling was scored from 0 (pen is clean) to four ( $\geq$ 75% of the pen is soiled). Average category score was calculated per group.

Animal behavior was scored by scan sampling after a 20-min habituation period to the observer. The animals were always observed live by the same trained assessor during 10 observation moments per pen, each lasting 1 min (Aluwé et al., 2016). Behavior categories were inactive, eating/drinking, moving, interaction, manipulation, playing, ear or tail biting, aggression, mounting, and anogenital sniffing (Table 3). The

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#### Table 3

Ethogram of recorded behaviors (Sus scrofa).

Behavior	Definition
Inactive	Pig is sleeping (eyes closed) or lying down
Eating/drinking	Pig is eating or drinking at feeding tray or drink nipple
Moving	Pig is walking around without exhibiting any other behavior
Interaction	Pig is sniffing other pig without showing aggressive behavior
Playing	Pig is playing with another pig without showing aggressive
	behavior
Manipulation	Pig is manipulating/biting distraction material, feeding trough, pen wall, etc.
Ear or tail biting	Pig is biting the ear or tail of another pig
Aggression	Thrusting by head-knocking or biting in the air, pushing another pig away, biting or lifting another pig
Mounting	Pig (attempts) to place front legs over front or back end of other pig and (attempts) to copulate
Anogenital sniffing	Nose of pig is in 5 cm proximity to anogenital zone of other pig

number of pigs per pen displaying the various types of behavior during each of the 1 min observation moments was recorded and average percentage of time spent during the observation moments per observation category was calculated per group.

#### Measurement on the day of slaughter

Information on the duration of loading, transport, and time spent in lairage were requested from the three participating slaughterhouses based on time at arrival at the farm, time at start of transportation and time of unloading which is routinely registered by the drivers. Carcass weight and lean meat percentage were also requested from the slaughterhouse. Carcass lesions were recorded by one single trained assessor after dehairing. Both sides of the carcass were scored on a scale from 0 to three, with score 0 representing  $\leq 8$  lesions/side and score three representing  $\geq 25$  lesions/side (Wesoly et al., 2014). The date of slaughter was used to construct a season variable. The average temperature on the day of slaughter was consulted in an online database (National Centers for Environmental Information, 2020), as well as the hours of daylight (US Naval Observatory, 2020).

#### Boar taint detection

All neckfat samples were evaluated by three trained panelists (ILVO employees) using the hot iron method (Heyrman et al., 2017). These three trained panelists (training according to Heyrman et al., 2017) participated based on their availability from an olfactory panel of six, hence the three trained panelists varied between but not within slaughter batches. Neckfat samples were mostly evaluated on the day of slaughter or the day after and kept at 4 °C before and in between evaluations (this was the method used for  $n = 14\,994$ ). Scoring was done on a five-point scale (0 = no taint, one = light taint, two = fair taint, three = strong taint, four = very strong taint) by each trained panelist independently and blind to each other. A sample was considered tainted when the final mean score was equal to or exceeded 1.5.

### Quality control

Olfactory evaluation is performed by people who are selected based on their sensitivity to androstenone and trained to evaluate boar taint in fat. In studies, olfactory boar taint is often compared to chemical analysis to determine panel performance and set the relevant cutoffs of the boar taint compounds (Mathur et al., 2012; Meier-Dinkel et al., 2015). For all entire male pigs included in the study, olfactory boar taint analysis was performed and for a selection of entire male pigs, boar taint compound concentrations were determined to compare to the performance Animal xxx (2021) xxx

of our trained olfactory panel and set the cutoff score for olfactory boar taint.

A selection of stored samples (balanced per farm and per slaughter batch) that received a final median score of 0 (n = 97), 1 (n = 98), 2 (n = 99), 3 and 4 (n = 100) were chemically analyzed for AND, SKA, and IND using HPLC-Orbitrap-MS (Bekaert et al., 2012a). A receiver operating characteristic (ROC) analysis was done to determine the sensory cutoff score to be used as a determinant for boar taint status per slaughter batch. This was done by choosing a cutoff score from the ROC curve were sensitivity and specificity were considered most optimal (Trautmann et al., 2016). If we consider chemical analysis as the reference method and olfactory assessment as a predictor sensitivity was 0.72 and specificity was 0.67 at a final mean score from the olfactory panel  $\geq 1.5$  (Fig. 2). This was considered as the most suitable tradeoff between sensitivity and specificity. Similar values for sensitivity (0.16 to 0.61) and specificity (0.97 to 0.82) have been applicable to trained panels used in other studies (Mathur et al., 2012; Meier-Dinkel et al., 2015).

### Data analysis

All data analyses were conducted in R (R Core Team, 2013) using the Ime4 package (Bates et al., 2015). The olfactory boar taint prevalence of the 1st and 3rd quantile of each potential risk factor was calculated to conveniently present the results (Table 1) (these quantiles were in no way involved in the statistical analysis however). All potential risk factors were analyzed for their relation to olfactory boar taint in separate mixed logistic regression models (1 = positive for boar taint, 0 = negative for boar taint). The potential risk factor was used as predicting variable, and farm and slaughter batch nested within farm as random effects. Further details on the model are provided in the supplementary material S1.

To analyze on which observational level there was the most variation in olfactory boar taint, a random effects model with olfactory boar taint as predicted variable and farm and slaughter batch nested within farm as random effects was used.



**Fig. 2.** Receiver operating characteristic (ROC) analysis for the selection of neckfat samples (n = 394) from entire male pigs from 23 farms. Sensitivity and specificity are shown for each possible olfactory cutoff score compared to exceeding the cutoff levels for one or more of the chemical boar taint compounds (androstenone (cutoff = 2.0 µg/g), skatole (cutoff = 0.25 µg/g), and indole (cutoff = 0.15 µg/g)). Olfactory score is the mean score of three trained panelists who scored the fat samples on a scale from 0 to 4. Based on this ROC curve, a cutoff score of 1.5 for olfactory boar taint was considered most optimal.

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#### Results

#### Boar taint prevalence

The average olfactory boar taint prevalence over all farms was 1.80  $\pm$  0.83%. The minimum and maximum range between slaughter batches within farms was 0.31 and 9.10% (Fig. 3).

The random effect shows that there is slightly more variation within farms (SD = 0.373) than between farms (SD = 0.289), there are, however, no statistical tests available for this hypothesis, so these findings are only indicative.

### Potential risk factors

The results from the univariable models relating each potential risk factor to olfactory boar taint are summarized in Table 1 to present the results of all continuous variables, and Table 2 presents the results of all factorial variables.

### Social hierarchy and puberty attainment

Slaughter batches where entire male pigs had more aggressive behavior (Fig. 4(a)) and batches that had higher average skin lesion scores in the compartment on the farm (Fig. 4(d)) had significantly higher olfactory boar taint (P < 0.001 and P = 0.002 respectively, Table 1).

Slaughter batches with more anogenital sniffing behavior (Fig. 4(b)) and batches with a higher percentage of pens with gilts (Fig. 4(c)) in the compartment had a trend for higher olfactory boar taint (P = 0.092 and P = 0.063, respectively, Table 1).

#### Housing

Slaughter batches with floors with smaller slat width (Fig. 4(e)) had significantly higher olfactory boar taint (P = 0.033, Table 1). Also, slaughter batches where entire male pigs where kept in pens with 4 open sides had significantly higher olfactory boar taint than in pens with two open sides, with 0, 1, and 3 open sides being intermediate (Fig. 4(m)) (P = 0.005, Table 2).

#### Health

Slaughter batches with less mortality (Fig. 4(f)) had significantly higher olfactory boar taint prevalence (P = 0.018, Table 1).



**Fig. 3.** Boxplots of the boar taint prevalence's (scored by a trained olfactory panel) of each slaughter batch per farm (n = 22) ordered by increasing average boar taint prevalence. The number of slaughter batches per farm are indicated by n(batch).

### Preslaughter conditions

Slaughter batches where entire male pigs stayed less time in lairage (Fig. 4(g)) before slaughter had significantly higher olfactory boar taint (P = 0.010, Table 1). On animal level, entire male pigs with higher skin lesion scores as scored after slaughter (Fig. 4(i)) had significantly higher olfactory boar taint (P < 0.001, Table 1).

A longer fasting period (Fig. 4(h)) slaughter was associated with more olfactory boar taint (P = 0.098, Table 1).

### Season

Slaughter batches slaughtered on colder days (lower average outside temperature) (Fig. 4(j)) had a trend for higher olfactory boar taint (P = 0.098, Table 1). More generally, olfactory boar taint was significantly higher for slaughter batches from December to May compared to from September to November, with intermediate boar taint prevalence from June to August (Fig. 4(n)) (P = 0.005, Table 2).

#### Feed

Slaughter batches fed a finisher diet with a lower CP level (Fig. 4(k)) had higher olfactory boar taint (P = 0.028, Table 1).

#### Carcass composition

Entire male pigs with lower lean meat percentage (Fig. 4(1)) had significantly higher olfactory boar taint (P < 0.001, Table 1).

#### Discussion

### Study limitations

It was not possible to do a multivariable analysis of the data. Several attempts were made to construct a multivariable model, but this could not be performed due to limitations that are inherent to some observational studies and boar taint research in general. First, when building a multivariable model (either with a forward, backward, or stepwise method), it is necessary to always use the same data i.e. to start from a data set where none of the variables contain missing values, this left us with a data set that was far too small to build this model. Second, we tried the same but starting from a data set with only the variables that came forward as significant from the univariable model, this still led to the same problem. Lastly, we attempted do construct several smaller models (two variables each) that we suspected could show interaction effects, but most of such models failed to converge. All these limitations are consequences of unbalanced and incomplete data (as this can be difficult to control in observational studies, in our case we followed up all farms keeping entire males we could find; hence we had no control over balancing variables; furthermore, some data were not available on certain farms). The model was also further complicated by the generally low prevalence of boar taint (leading to a high number of "0" values in the depended variable). Routine reliable evaluation of boar taint and boar taint compound in the slaughterhouses would boost this kind of studies and may make it possible to do multivariable analysis. On the other hand, these limits themselves might be less of an issue once more farmers make the transition to keeping entire males, researches would then have more opportunity in designing their studies to meet the needs of the desired statistical analysis to perform afterwards. This does not mean, however, that the univariable analysis in entirely without merit, it merely must be kept in mind that the full picture is likely more complex due to possible interactions.

### Boar taint prevalence

The overall mean olfactory prevalence was 1.8%; other studies found a prevalence of 3 to 4% (van Wagenberg et al., 2013; Aluwé et al., 2014). We also observed variation in boar taint prevalence between farms and

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within farms, so boar taint prevalence cannot be said to be solely farm specific. Further evaluation of the factors that vary between slaughter batches is therefore of interest.

### Potential risk factors

#### Social hierarchy and puberty

No effect of estimated attainment of puberty (based on the farmer observations of increased aggressive and sexual behavior) was found. We found more aggressive behavior in the weeks before slaughter for slaughter batches with higher risk for olfactory boar taint. Also, the average skin lesion score recorded in this period was higher for these slaughter batches. We therefore hypothesize that groups of entire males that are more engaged in fighting behavior during the last stages before slaughter can thus be expected to have a higher boar taint prevalence (Parois et al., 2016). Previous studies indicate that dominant pigs have increased plasma testosterone concentrations as well as higher fat androstenone levels (Giersing et al., 2000). For the less dominant animals in a pen, fat androstenone is also increased by the presence of entire males with higher fat androstenone (Giersing et al., 2000). These lighter, less dominant pigs (with lower AND and therefore SKA become the victim of more aggressive behavior resulting in more skin lesions (bullying) (Giersing et al., 2000). A negative correlation between skin lesions and AND and SKA at animal level could also be expected, as it has been shown previously (Bekaert et al., 2012a).

Ano-genital sniffing behavior also showed a trend for higher olfactory boar taint rating. Mounting behavior showed the same direction of effect but was nonsignificant, this may suggest a possible link between sexual behavior and boar taint. We also found that slaughter batches with a higher percentage of pens with gilts showed a trend for higher risk for boar taint. The effect of gilts in the same pen or compartment varies across studies. Some studies have not found significant differences between entire male pigs which had or had no visual contact with gilts in the same compartment (Fàbrega et al., 2011). Other studies did show lower SKA concentrations (Andersson et al., 1999), but higher AND concentrations (Zamaratskaia et al., 2005) for entire male pigs kept in mixed sex pens compared to entire male pigs kept in single sex pens. The hypothesis is that presence of gilts stimulates puberty attainment.

These results for social hierarchy and aggression suggest that any measure, i.e. management as well as genetic selection to reduce aggressive behavior in the compartment could be useful to reduce boar taint prevalence. Off course, this is also interesting from an animal welfare point of view and is therefore worthwhile of further investigation.

#### Housing

The stocking rate was not significantly linked with boar taint risk. In previous studies, lower stocking rate has been associated with lower aggression (Turner et al., 2000), but it may also reflect lower competition in the pen. All farms included in our study had at least chains in each pen as distraction material. The slaughter batches which had supplementary distraction material were not found to have significantly lower risk for boar taint, suggestive of genetic factors and potentially other environmental factors. The presence of enrichment material has been shown to reduce aggressive behavior in barrows and gilts (Simonsen, 1990), but this was not confirmed in our study.

None of the potential risk factors associated with fouling and cleaning of the pens was found to be significantly linked with boar taint. For animal and pen fouling as observed in the weeks prior to slaughter, the direction of the observed effect was numerically even opposite to what could be expected.

Between entire male pigs kept on partly or fully slatted floor, there was no significant difference in boar taint risk. Slat width was the only floor related risk factor that showed a significant link, with lower slat width associated with higher boar taint risk. Which is strange as slot/ slat ratio did not show a significant effect on boar taint, while mainly this variable is considered influential on pen soiling (Vermeij et al., 2009). It has to be kept in mind that the number of open sides is associated with floor type as only fully slatted floors had three or four open sides. The significant results for this risk factor might be explained by the low number of animals in the group with four open sides, which shows the biggest difference.

It was also hypothesized that ventilation type may play a role in SKA accumulation because it clears SKA from the air, lowers inside temperature and relative humidity (Lundström et al., 1988; Hansen et al., 1994). However, we also did not find significant differences for boar taint risk between ventilation types.

#### Health

Neither the occurrence of diarrhea (no: 2.0 vs yes: 2.9%) nor the use of antibiotics at any time during rearing by any method (no: 2.3 vs yes: 1.9%) were significantly confirmed as related risk factors with boar taint risk in our study, although numerically in line with expectations. Higher SKA concentrations in fat have been found for entire male pigs who suffered from dysentery, possibly due to mucosal damage leading to increased supply of tryptophan or reduced liver catabolism (Škrlep et al., 2012). Also infections with *Lawsonia intracellularis* may increase SKA production by increasing cell wall turnover in the gut (Visscher et al., 2018). Antibiotics in the feed have been shown to lower SKA concentrations in fat by reducing the number of micro-organisms in the gut (Hansen and Larsen, 1994).

Only lower mortality was significantly linked with higher risk for boar taint. This is a puzzling result, but one possible hypothesis is that it might be related to health or aggressive behavior.

#### Preslaughter

Besides the conditions during fattening, aspects just before slaughter may also influence boar taint levels, likely due to elevated AND concentrations. In the current study, we found that slaughter batches were kept shorter time in the lairage had a higher risk for boar taint, possibly these pigs did not have time to recover from the stress experienced during transport. However in a previous study, no effect was found (Heyrman et al., 2018). Entire male pigs with higher skin lesion score after slaughter also had a higher risk for boar taint, further indicating that entire male pigs who engage in more aggressive behavior before slaughter have a higher risk of exhibiting boar taint, although the effect is rather small. It has been found that aggressive behavior during transport and lairage due to mixing and general stressful conditions of transport can lead to increased skin lesions recorded after slaughter (Faucitano, 2000). Longer transport durations, longer pre-unloading time (Wesoly et al., 2014), longer time in the lairage, and increase in skin lesions have been linked with higher boar taint prevalence in previous studies (Heyrman et al., 2017). These results further point to the importance of effects on boar taint playing in these final moments

**Fig. 4.** Relation of factors measured at slaughter and boar taint (by olfactory panel) for a subsample (n) of entire male pigs for the univariate model: Regression line of P(Boar taint) in function of (a) aggressive behavior; (b) ano-genital sniffing; (c) percentage of mixed pens; (d) skin lesion score in compartment; (e) slat width; (f) mortality; (g) duration in lairage; (h) fasting period (h: hours); (i) skin lesion score after slaughter; (j) average outside temperature on slaughter date; (k) CP; (l) lean meat percentage. Bar plots of factorial variables and their relation with P(Boar taint) (m) pen separation; (n) season (DJF: December, January, February – MAM: March, April, May – JJA: June, July, August – SON: September, October, November). Dots and dotted line (summarized data not involved in the analysis): mean values and standard error for P(Boar taint) with each variable divided into equidistant categories, each dot indicates the P(boar taint) for the average of the corresponding category. P(boar taint): chance that the olfactory panel would consider an entire male carcass to be tainted, a, b indicate significance at *P* < 0.05.

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before slaughter. These effect may be linked to stressful and aggressive conditions during transport (Wesoly et al., 2014). Higher aggressive behavior has been found for pigs fasting for 24 h compared to pigs fed freely (Kelley et al., 1980), this might explain the trend for a positive link between fasting duration and boar taint risk as a consequence of aggressive behavior exacerbated by time off feed. A future experiment looking at the evolution of boar taint compounds during these different stages during transport using biopsies could further clarify these results.

#### Season

The average outdoor temperature on the day of slaughter showed a trend for a negative link with the risk for boar taint. More generally, olfactory boar taint was significantly higher for slaughter batches from December to May compared to from September to November, with intermediate boar taint prevalence from June to August. AND is produced in the testes, while SKA and IND are produced by micro-organisms in the gut, AND further inhibits SKA catabolism in the liver leading to higher deposition (Rius and García-Regueiro, 2001). It has been assumed that pigs show more pubertal development and testicular activity in autumn as a result of the shortening of days (Claus et al., 1983), suggesting higher AND levels in winter as well as in autumn. At increased temperature, however, the liver metabolism of skatole may be less, resulting in increased SKA concentrations in fat linked to a proposed ska gene (Lundström et al., 1994; Deslandes et al., 2001), suggesting higher SKA in summer and autumn. This may imply that the seasonal effect of temperature on pubertal development might run contrary to the direct effect on SKA metabolism as related to boar taint. On the one hand, pubertal development and thus AND increases in the fall and winter seasons, while on the other hand, SKA metabolism is impaired and thus SKA increases in the spring and mainly summer (i.e. warmer) seasons. More insight in the effects of season and temperature could help slaughterhouses plan in which periods more tainted carcasses can be expected and to seek alternative markets.

#### Feed

Slaughter batches fed a diet with a lower protein content had a higher risk for boar taint. This is in line with previous studies which showed that lower CP levels have been linked with higher SKA production. One explanation being that higher CP diets results in higher ileal digestibility of protein resulting in less protein and tryptophan available for SKA production. Another explanation is that higher CP levels lead to more lean than fat growth and consequently lower boar taint levels (Lin et al., 1992). Further high energy content of the feed leads to higher skatole production because it results in higher IGF-1 which results in a higher mitotic and apoptotic rate of the enterocyte wall, and this in turn leads to more tryptophan being available (Claus and Raab, 1999).

Feed composition can influence the concentration of SKA in adipose tissue through influencing either SKA production, transit time, absorption in the gut, or liver metabolism (Zamaratskaia and Squires, 2009). Several carbohydrates (i.e. raw patato starch, lupines, chicory, and inuline (Chen et al., 2007; Nielsen et al., 2007; Kjos et al., 2010)) have been found to influence SKA production by changing the microflora in the gut. Undigested carbohydrates can also lower SKA absorption by reducing the transit time. Water content can also influence SKA absorption (Zamaratskaia and Squires, 2009). A higher crude fiber content has been linked with higher SKA production due to more active microflora (Lin et al., 1992), one other study found no such effect (Oeckel et al., 1998).

Recent research has also pointed to the possibility of lowering AND concentration via plant metabolites and flavonoids working on liver catabolism (Urbanová et al., 2016).

### Carcass composition

A negative correlation between lean meat percentage and boar taint has been found earlier (Walstra et al., 1999) and this was again confirmed in this study: entire male pigs with a higher lean meat percentage had lower risk for boar taint. Lean meat percentage has been positively linked with the amount of unsaturated fatty acids in fat (Wood et al., 2008). It has been suggested this may possibly increase the release of AND and SKA from fat tissue in vivo (Rius et al., 2005). It is also hypothesized that this may also negatively influence the release of AND and SKA from fat tissue after heating and thus lower olfactory perception directly (Rius et al., 2005). Another hypothesis is that cytochromes in the liver are used both in steroid (AND) and lipid synthesis and metabolism (Rius et al., 2005; Mörlein and Tholen, 2014) and thus influence both boar taint compounds and fat composition. The underlying mechanism is however not clear.

#### Age or weight

In the current study, we did not find a significant link with boar taint risk within the range of commercial slaughter weights  $(95 \pm 12 \text{ kg})$ . For carcass weight, a low but significant positive correlation has been found between with boar taint in a large scale study ( $n = 4\,313$ , range = 48 to 107 kg) (r = 0.10) (Walstra et al., 1999). In a small study (n = 33), with live weight ranging from 106 to 157 kg, higher correlations have been found for AND (r = 0.43) as well as SKA (r = 0.46), which was linked to pubertal development (Babol et al., 2002). These results indicate that at least at the current range of commercial slaughter weights in Flanders, the effect of weight on boar taint risk is not relevant.

#### Breed

We were not able to identify significant differences in boar taint risk between sow lines or type of Piétrain sire, despite numerical differences. Effect of weight, feed intake, growth, lean meat percentage, etc. on boar taint compounds may be breed dependent and more clearly also boar taint levels differ between breeds. Also genotype can play a role as shown for the MC4R gene (Van Den Broeke et al., 2015). Several studies have shown breed differences in boar taint prevalence, mainly due to differences in puberty attainment and consequently AND concentrations. Also differences in SKA concentration between breeds have been found (Zamaratskaia and Squires, 2009).

#### Conclusion

We can generally conclude that measures that reduce aggression and stress groups of entire male pigs may have the potential to lower boar taint prevalence in commercial setting, which would likely also benefit the welfare of the pig. The same might be said for sexual behavior, though less convincingly from this study. This applies to the final weeks on the farm but possibly also to the period before slaughter. Furthermore, boar taint prevalence is generally higher in winter than in summer, this can be kept in mind from a marketing perspective. Further negative effect on boar taint of CP content seems relevant but requires further experiments to find a causal relation. The same can be said for the negative effect on boar taint of lean meat percentage which is expected from literature. Previously found effects of slaughter weight were not found here on the other hand. A widely applied, comparable on-line boar taint detection system combined with a high number of entire male pigs originating from different farms would likely create more suitable conditions to shed light on any possible interaction effects between potential risk factors when performing similar future field studies.

#### Supplementary materials

Supplementary data to this article can be found online at https://doi. org/10.1016/j.animal.2020.100141.

#### **Ethics approval**

Not applicable.

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# Data and model availability statement

None of the data were deposited in an official repository.

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### Author contributions

E. Heyrman, M. Aluwé, S. Millet, F.A.M. Tuyttens, S. Janssens, N. Buys: Conceptualization. E. Heyrman, M. Aluwé, B. Ampe: Methodology. E. Heyrman: Formal analysis. E. Heyrman: Investigation. J. Wauters, L. Vanhaecke: Resources. E. Heyrman, M. Aluwé: Writing – original draft. E. Heyrman, M. Aluwé, S. Millet, F.A.M. Tuyttens, S. Janssens, N. Buys, J. Wauters, L. Vanhaecke: Writing – review & editing. M. Aluwé, S. Janssens, N. Buys: Supervision.

#### **Declaration of interest**

None.

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