1	The effects of handling and group size on welfare of pigs in lairage and their influence
2	on stomach weight, carcass microbial contamination and meat quality
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5	C. Rabaste <sup>1,2</sup> , L. Faucitano <sup>1*</sup> , L. Saucier <sup>2</sup> , P. Mormède <sup>3</sup> , J.A. Correa <sup>4</sup> , A. Giguère <sup>1</sup> , and R.
6	Bergeron <sup>2</sup>
7	
8	<sup>1</sup> Agriculture and Agri-Food Canada, STN Lennoxville, P.O. Box 90, College Street,
9	Sherbrooke, Québec, Canada J1M 1Z3
10	<sup>2</sup> Département des sciences animales, Université Laval, Pavillon Paul-Comtois, Sainte-Foy,
11	Québec, Canada G1K 7P4
12	<sup>3</sup> Laboratoire de Neurogénétique et Stress, Institut National de la Recherche Agronomique,
13	Université Victor Segalen Bordeaux 2, 146 Léo-Saignat, 33077 Bordeaux Cedex, France
14	<sup>4</sup> F. Ménard Inc., 251, Route 235, Ange-Gardien, Québec, Canada JOE 1EA
15	
16	
17	*Corresponding author:
18	Luigi Faucitano
19	Tel.: +1 (819) 565-9174
20	Fax: +1 (819) 564-5507
21	Email: <u>Faucitanol@agr.gc.ca</u>
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23	RUNNING HEAD: Preslaughter handling, welfare and pork quality

Abbreviations: CA, catecholamines; DL, drip loss; DM, dry matter; EC, electrical
 conductivity; GC, glucocorticoids; LT, Longissimus thorasis; RH, rough handling; GH,
 gentle handling; TAM, total aerobic mesophilic

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5 Rabaste, C., Faucitano, L., Saucier, L., Mormède, P., Correa J. A., Giguère, A. and Bergeron, 6 R. 2006. The effects of handling and group size on the welfare of pigs in lairage and their 7 influence on stomach weight, carcass microbial contamination and meat quality. Can. J. 8 Anim. Sci. xx:---.At unloading and on the way to stunning, 800 barrows were submitted to 9 either gentle handling (GH: slowly with a plastic board or whip) or rough handling (RH: 10 quickly with an electric prod). Pigs were kept in large or small groups (30 or 10 pigs) during 11 lairage. Compared to GH, RH increased climbing (P < 0.05), slipping (P < 0.01) and turning 12 around (P < 0.001) behaviours during unloading, and climbing (P < 0.05) on the way to stunning. RH also reduced drinking behaviour during lairage (P < 0.01). Pigs kept in large 13 14 groups were observed more often standing (P < 0.05) and fighting (P < 0.001) than pigs kept 15 in small groups, but in contrast, had a slightly lower level of urinary cortisol at slaughter. 16 Stomach weight and microbial contamination at slaughter were not affected by treatments. 17 RH tended to increase skin bruise score on the carcass (P < 0.06) and produced more 18 exudative meat (P < 0.05). In conclusion, the response of pigs to the two specific stressors 19 applied prior to slaughter in this study does not seem to contribute to stomach weight 20 variation at slaughter, but it does influence pork quality.

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Key words: Pigs, pre-slaughter handling, group size, stress, stomach weight, microbial
contamination, behaviour, meat quality

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1	Rabaste, C., Faucitano, L., Saucier, L., Mormède, P., Correa J. A., Giguère, A. et Bergeron,
2	R. 2006. Effets de la manipulation et de la taille du groupe sur le bien-être des porcs à
3	l'abattoir et leur influence sur le poids des estomacs, la contamination microbienne de la
4	carcasse et la qualité de la viande. Can. J. Anim. Sci. xx: Au déchargement et en route
5	vers l'anesthésie, 800 porcs mâles castrés ont été soumis à des manipulations douces (GH:
6	lentement avec un panneau de plastique ou un fouet) ou rudes (RH: rapidement avec un
7	aiguillon électrique). Pendant la période de repos, les porcs ont été maintenus en grands ou en
8	petits groupes (30 vs 10). Les RH, comparés aux GH, ont augmenté au déchargement les
9	chevauchements ( $P < 0.05$ ), les glissades ( $P < 0.01$ ) et les demi-tours ( $P < 0.001$ ) et sur la
10	route vers l'anesthésie, les chevauchements ( $P < 0,05$ ). Les RH ont également réduit les
11	abreuvements pendant l'attente ( $P < 0,01$ ). Les porcs maintenus en grands groupes ont été
12	plus souvent observés debout ( $P < 0,05$ ) et en combat ( $P < 0,001$ ) que les porcs maintenus en
13	petits groupes, mais en revanche, ils ont eu une concentration de cortisol urinaire légèrement
14	plus faible à l'abattage. Le poids des estomacs et la contamination microbienne à l'abattage
15	n'ont pas été affectés par les traitements. Les RH ont eu tendance à avoir un score de lésions
16	sur la peau plus élevé ( $P < 0,06$ ) et à produire des viandes plus exsudatives ( $P < 0,05$ ). En
17	conclusion, la réponse des porcs aux deux facteurs de stress spécifiques appliqués avant
18	l'abattage dans cette étude ne semble pas contribuer à la variation du poids des estomacs,
19	mais influence la qualité de la viande.

Mots clés: Porcs, manipulation pré-abattage, taille du groupe, stress, poids des estomacs,
contamination microbienne, comportement, qualité de la viande

#### **INTRODUCTION**

2 Slaughtering animals with a full stomach is considered a high risk factor for meat safety, as 3 spillage of gut contents, due to more frequent inadvertent puncture of the stomach during the 4 dressing process, can lead to microbial cross-contamination between carcasses (Miller et al. 5 1997). To reduce the risk of puncturing the stomach, a feed withdrawal of 16-24 h before 6 slaughter has been recommended to reduce stomach size (Chevillon 1994). However, industry 7 reports and some studies have revealed a high variability in stomach weights at slaughter, 8 even among pigs that were subjected to the same fasting interval before slaughter (Guise et al. 9 1995; Turgeon 2003). According to Enck et al. (1989), stress increases intestinal motility, 10 resulting in a greater evacuation of the caecum and large intestine. It also increases the pH of 11 the stomach contents, favoring the survival, proliferation and release of fecal bacteria (such as 12 Salmonella), both towards the internal organs and the surrounding environment (Gregory 13 1998). Hence, the individual difference in the pig response to preslaughter stress might 14 contribute to stomach weight variation at slaughter.

15 Beside creating a reservoir of animals aimed at maintaining the constant speed of the 16 slaughter line, the function of lairage is to allow the animals to recover from the stress 17 endured during transport and unloading, and consequently to improve meat quality. Hence, a 18 lairage time of 2-4 h is recommended (Warriss 2003). However, these benefits can be lost if 19 lairage facilities are not well designed and poor handling procedures are applied (Hunter et al. 20 1994; Faucitano et al. 1998; van der Wal et al. 1999). The use of electric prods must be 21 limited in pig handling given their detrimental effects on welfare (flight behaviour, higher 22 heart rate and salivary cortisol level) and meat quality (Brundige et al. 1998; D' Souza et al. 23 1998; Jongman et al. 2000). However, the high slaughter speed of modern abattoirs is often 24 assured by the indiscriminate use of this handling device (Geverink et al. 1996; Jones 1999).

1 Mixing unfamiliar pigs inevitably causes some fighting which causes skin bruises and 2 poor pork quality (Jones et al. 1994; Warriss 1996). However, it is a common practice to mix 3 large groups of pigs (up to 90 per pen) either because of the lack of adequate holding facilities 4 (adjustable pen size) or because a change in this practice is not perceived by the abattoir 5 managers as economically important. To limit fighting and help pigs rest and recover from 6 transport stress, the current recommendations are either to keep pigs in small groups (10-15 7 pigs) or to mix very large groups (up to 200 pigs) in the lairage pen (Grandin 1990; 8 Christensen and Barton-Gade 1997). However, as emphasized by Warriss (2000), the degree 9 of fighting during lairage is rather related to the harshness of previous handling than on group 10 size. Turner et al. (1999) showed that group size influences drinking behaviour, pigs in large 11 groups (60) spending significantly less time drinking than pigs in smaller groups (20). Hence, 12 it may be hypothesized that the difference in drinking rate between groups of pigs varying in 13 size may contribute to stomach weight variation at slaughter.

The aim of this experiment was thus to determine, under commercial conditions, the effects of gentle *vs* rough handling practices and large *vs* small group size in lairage on behaviour, stomach weight, microbial carcass contamination and meat quality variation of pigs.

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#### **MATERIALS AND METHODS**

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#### 21 Animals and Treatments

Ten batches of 80 crossbred barrows (synthetic line provided by F. Ménard, Ange-Gardien, QC; average weight:  $108.1 \pm 4.1$  kg) were randomly selected, identified with an individual mark on their back and side at the same farm (one batch per week), fasted (16 h prior to loading) and transported in separate compartments of 20 pigs each for 1 h to a commercial 1 abattoir. At unloading, pigs from each batch were randomly distributed between two handling 2 treatments (RH: rough and GH: gentle), and two group sizes (10 and 30) were formed in the 3 lairage pens. Pigs handled roughly were moved as quickly as possible with an electric prod, 4 whereas pigs handled gently were moved slowly with a plastic board during unloading, and a 5 whip (used to tap on the back, only when necessary) on their way to the restrainer. Pigs 6 handled roughly each received one electric shock upon unloading from the truck, when 7 entering the lairage pen, and on their way to the restrainer (Fig. 1). During unloading and 8 when moved towards the restrainer, all animals were handled in groups of 10.

In lairage, pigs from both large and small groups were kept at a stocking density of 0.59 m<sup>2</sup>/pig per pen, with a ratio of one water nipple per 10 pigs. Pigs were showered for 13 min from the start of the lairage period, and for 15 min before being slaughtered. All pigs were kept in lairage for 3 h and fasted for a total of 20 h. Animals were cared for according to a recommended code of practice (Agriculture and Agri-Food Canada 1993) and to the guidelines of the Canadian Council on Animal Care (1993), with the exception of the use of a whip in the stunning raceway, which reflects commercial practice."

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#### 17 Behavioural Recordings

18 During unloading from the truck, the frequency of climbing, slipping and turning around was 19 recorded on all groups of 10 pigs belonging to the small group size treatment, and on 20 randomly selected subgroups of 10 pigs from each large group size treatment (total sample 21 size of 400 pigs). During the first hour of lairage, standing, lying down, drinking and 22 aggression were recorded over four periods of eight minutes on the same animals. Aggressive 23 behaviours were classified as fighting and agonistic acts (bites and head knocks). The number 24 of pigs standing and lying down was recorded by scan sampling at 2 min intervals, while the 25 frequency of drinking and aggressive behaviours was recorded continuously. When pigs were moved to the restrainer, the frequency of climbing and turning around was recorded but only on pigs that had been kept in groups of 10 during lairage. Behavioural variables were noted by two observers placed along the resting pens and the raceways. Over the experiment, the observers alternated between treatments combinations for a more reliable behavioural evaluation.

6

#### 7 Physiological Measures

8 Urine samples were collected directly on the slaughter line from the bladders of the same 9 animals observed during lairage. Following the addition of HCl 6 M (1% of urine volume) as 10 preservative, samples were immediately frozen (- 45° C) pending analysis of urinary free 11 corticoids (cortisol and cortisone) and catecholamines (CA) (noradrenalin, adrenaline and 12 dopamine). Corticoids were measured by high pressure liquid chromatography (HPLC) 13 coupled with UV detector after extraction on reverse phase columns (Hay and Mormède 14 1997a). CA were measured by HPLC coupled with an electrochemical detector after 15 extraction on cationic columns (Hay and Mormède 1997b). Creatinine was measured by a 16 colorimetric quantitative method (Procedure 500, Sigma Diagnostics, Saint-Quentin-Fallavier, 17 France) to correct for urine dilution.

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#### 19 Stomach Weight and Content Composition

Stomachs were removed from 400 carcasses on the dressing line. They were identified and stored at 4°C until they were weighed, full and emptied of their content. Stomach content weights were expressed on a wet weight basis and the state of fasting was assessed according to the criteria set by Chevillon (1994). The contents were mixed and a qualitative assessment of contents was made (sawdust from the truck, liquid or feed). The percentage of dry matter (DM) was calculated after lyophilization at 50°C.

#### 1 Microbial Analysis on the Carcass

2 Carcasses from all animals that had been observed during unloading and lairage were 3 sampled. Swabbing was carried out using a sterile sponge kept in a Whirl-pak<sup>™</sup> sampling 4 bags (#B01245, Nasco, Fort Atkinson, WI). Ten milliliters of diluent constituted of 0.1% 5 peptone water supplemented with 1% Tween 80 was added to each bag and samples were 6 maintained on ice for transportation and kept at 4 °C until analysis. The sponge was applied 7 on the internal rib cage, on the briskets and on the top of the two front feet for a total approximate surface of 983 cm<sup>2</sup>. Escherichia coli, coliforms and total aerobic mesophilic 8 9 (TAM) counts were performed using hydrophobic grid membrane techniques described by 10 Gill and Jones (2000) using the Spreadfilter from Filtaflex Ltd. (Almonte, ON). Samples were 11 stomached for 2 min prior to cell enumeration. Serial dilutions were filtered and membranes (ISO-GRID<sup>TM</sup>, Neogen, Lansing, MI) were incubated on appropriate agar medium. TAM 12 13 counts were performed on Tryptic Soy Agar (TSA; Becton Dickinson, Missisauga, ON) 14 incubated at 35 °C for 48 h and colonies were stained with a 0.1% solution of 15 Triphenyltetrazolium chloride (Sigma Aldrich, Oakville, ON) for 15 min. Coliforms were 16 enumerated on Lactose Monesin Glucuronate agar (LMG; QA Life Sciences, San Diego, CA) 17 after an incubation of 24 h at 35°C. The filters were then transferred on buffered 4-18 methylumbeliferyl-β-D glucuronide agar (BMA; QA Life Sciences) and incubated for 2 h at 19 35°C for E. coli enumeration after illumination of the grid with a long-wave UV light. Grids 20 were photographed (Coolpix995, Nikon Corp., Tokyo, Japan) and the most probable number 21 (MPN) was determined using the software HGMF Interpreter (Filtaflex Ltd). Pieces of meat 22 trimmed off on the processing line for visual contamination were recorded for each carcass.

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#### 24 Skin Bruises and Meat Quality Measurements

1 Skin bruises were assessed in the cooler immediately after dressing using a photographic 2 scale from 0 (none) to 5 (severe; MLC 1985). Muscle pH was assessed at 45 min (pH<sub>1</sub>) and 3 24 h (pHu) post mortem with a pH meter (Oakton Instruments Model pH 100 Series, Nilis, 4 IL) fitted with a Cole Parmer spear type electrode (Cole Palmer Instrument Company, Vernon 5 Hills, IL) and an automatic temperature compensation probe by insertion in the *longissimus thorasis* (LT) muscle between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs (Canadian grading site). At 24 h post 6 7 mortem the following measures were taken from the LT muscle in the carcass grading site 8 region: electrical conductivity (EC) by insertion of the Pork Quality Meat probe (PQM-I-9 INTEK, Gmbh, Aichach, Germany), subjective color score using the Japanese Color 10 Standards (JCS) ranging from 1 = pale to 6 = dark color (Nakai et al. 1975), light reflectance 11 using a Minolta Chromameter CR 300 (D65 light source with 0° viewing angle geometry) 12 according to the reflectance coordinates (CIE L\*, a\*, b\*), and after exposing the muscle 13 surface during a one-hour blooming time. Drip loss (DL) was assessed on an adjacent chop 14 according to a modified "juice container" procedure (Christensen 2003). Briefly, three 15 cylindrical muscle cores were excised using a cork borer (25 mm diameter) from the chop and 16 weighed. Later on, the cores of muscle were placed into a DL plastic container (Christensen 17 Aps Industrivaengetand, Hilleroed, Denmark) and stored at 4°C. Forty-eight h after sampling, 18 the sample was removed from the container, carefully dabbed and weighed. DL was finally 19 estimated by calculating the difference between the initial and the final weight of the muscle 20 sample.

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#### 22 Statistical Analysis

All statistical analyses were performed using the SAS 9.1 software (SAS 2002), with each group of pigs as an experimental unit. Behavioural data recorded during unloading and handling towards the restrainer were analysed with a Wilcoxon-Mann-Whitney test. In

1 lairage, behavioural data were analysed with a Friedman test for continuous measures 2 (drinking and aggressive behaviours), and by analysis of variance with repeated measures for 3 postures (lying down or standing). Corticoids and catecholamines were analysed after log 4 transformation to correct for deviation from normality, which was assessed by a Shapiro-Wilk 5 test. These data were submitted to an analysis of variance according to a 2 x 2 factorial design 6 in complete randomized blocks. Behavioural and hormonal data were only analysed for 9 7 blocks, because observation conditions were different on the first week due to technical 8 problems. Stomach data were also submitted to analysis of variance, with the exception of 9 content type data, which were analysed by a Chi-square test. Meat quality data were 10 submitted to an analysis of variance according to a 2 x 2 factorial design in complete 11 randomized blocks. For carcass contamination level, all bacterial counts were transformed to 12 Log values and a Shapiro-Wilk test for normal distribution was applied. Log values of the 13 means (log A) for sets of counts were calculated from the formula Log A=  $\overline{x}$  + Log<sub>n</sub>10 14  $(SD^2/2)$  (Brown and Baird-Parker 1982). The Log value of the lowest detection level 15  $(Log_{10}(5) = 0.69897)$  was assumed for the calculation of  $\bar{x}$  and SD values for coliforms and 16 E. coli counts when no colony was detected on the grid. A Log value for the total number of 17 bacteria recovered (N) was calculated for each set of counts by adding the counts in each set 18 and calculating the Log of the sum. These Log values were submitted to an analysis of 19 variance with the option LIFEREG of SAS. The carcass trimmings taken on the dressing line 20 were analysed with a Fischer test.

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No interactions were found between handling quality and group size during lairage for 22 any of the variables under study. Thus data were pooled across treatments and discussed 23 according to the main effects of handling type and group size only.

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# RESULTS

#### 1 **Behavioural Observations**

During unloading from the truck, rough handling increased the frequency of climbing (*P* <</li>
0.05), slipping (*P* < 0.01) and turning around (*P* < 0.001) (Table 1). In the stunning chute,</li>
rough handling only increased the frequency of climbing (*P* < 0.05) (Table 1).</li>

5 In lairage, the pigs kept in large groups were observed more often standing (P < 0.05) 6 than pigs in small groups, which spent more time lying (P < 0.05). No significant differences 7 in postures were observed between RH and GH pigs (Standing:  $RH = 55.8 \% \pm 8.2$  and GH =55.7 % ± 8.6 and lying down: RH = 44.2 % ± 8.2 and GH = 44.3 % ± 8.6). RH pigs were 8 9 observed less often drinking than GH pigs (10.4  $\% \pm 5.3 \text{ vs}$  19.1  $\% \pm 9$ ; P < 0.01), whereas no 10 difference between groups sizes was recorded (group of 30: 13.6  $\% \pm 5.0$  and group of 10: 16 11  $\% \pm 8.2$ ). The pigs kept in large groups fought 10 times more than pigs in small groups (P < 12 0.001) and were more often observed in agonistic interactions (P < 0.05) (Fig. 3). The type of 13 handling used to drive pigs to the pen did not influence the frequency of fighting and 14 agonistic interactions during lairage. The frequencies of fighting were 6.4  $\% \pm 3.5$  and 4.8 %15  $\pm$  2.1, and the frequencies of agonistic interactions were 6.2 %  $\pm$  1.1 and 8.7 %  $\pm$  2.3, for pigs 16 handled roughly and gently, respectively. Overall, at the start of the lairage period, 70 to 90 % 17 of pigs were standing, but this proportion decreased to 10 to 30 % at the end of the first hour 18 (Fig. 4).

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#### 20 Physiological Measures

The type of handling had no impact on any physiological stress indicators in the urine at slaughter, except for the cortisol concentration (Table 2). Indeed, cortisol level increased (P < 0.05), although to a little extent, in the urine of pigs kept in small groups compared to those in larger groups.

#### 1 Stomach Weight and Content Composition

2 The handling quality and group size during lairage did not affect stomach weight (full and 3 empty) or DM of contents (Table 3). The number of stomachs containing liquid, sawdust or 4 feed was not different between treatments either (Table 4). However, most of the stomachs 5 evaluated in this study contained mainly liquid (76.5%). Only 17.7% and 5.8 % of stomachs 6 contained feed and sawdust, respectively. In addition, taking into account the criteria set by 7 Chevillon (1994) to assess the fullness of stomachs due to incorrect fasting ( $\leq 1.4$  kg for 8 stomach weight and  $\leq 500$  g for content weight), 24.6 % of the stomachs were full or partially 9 full. Of this proportion, 79.4 % contained mainly liquid.

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#### 11 Carcass Hygiene

12 The handling quality (rough vs gentle) and group size (10 vs 30 animals) applied during 13 lairage had no significant impact on the level of microbial carcass contamination whether it is 14 for the TAM, coliforms or *E. coli* counts (Table 5). The 400 total aerobic mesophilic counts 15 were all well below 3  $Log_{10}$  CFU/cm<sup>2</sup>, and coliforms and generic *E. coli* were all below 60 16 Log<sub>10</sub> CFU/cm<sup>2</sup>. Furthermore, no coliforms or *E. coli* were detected on a fair percentage of 17 the grids analysed. During evisceration, only one stomach was perforated. The number of pig 18 carcasses per treatment from which meat was trimmed off by the veterinary inspectors and the 19 abattoir employees on the dressing line because of visual contamination and defects was not 20 different between treatments (GH = 32, RH = 42 and group of 10 pigs = 42, 30 pigs = 31).

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#### 22 Skin Bruise and Meat Quality

Bruise score tended to be higher (P = 0.06) in RH pigs compared to GH ones (Table 6). The type of handling also influenced pork quality, pH<sub>1</sub> being lower (P < 0.01) and DL, EC and a\* (redness) values being higher (P < 0.05, P < 0.01 and P < 0.05, respectively) in the LT muscle</li>
 in RH.

Group size only affected the pH fall at 24 h post mortem, pHu value being slightly higher (*P*< 0.01) in the LT muscle from pigs kept in the larger group size.</li>

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#### DISCUSSION

There is evidence that a shock with an electric prod is more aversive to pigs than inhaling 90%  $CO_2$  (Jongman et al. 2000) and can cause bruises on the carcass and lead to poor meat quality (Faucitano 2001a; Hemsworth et al. 2002). For this reason, the use of electric goads must be very limited (shocks lasting < 2 sec; EC 1993). The results of this study revealed that the use of the electric prod as part of the rough treatment, although limited, produced panic within the group, increasing escape attempts at unloading and at the entrance into the stunning chute, thus making the group more difficult to handle.

The quality of handling from the unloading deck to the pen only had an effect on drinking behaviour during lairage. GH pigs drank about twice more often than RH pigs. Considering that GH pigs did not receive electric shocks during handling, it is possible that they were less stressed and adapted faster to their new environment than RH pigs. This result confirms the observation of Warriss (2000) about the effect of previous handling on the behaviour of pigs in the lairage pen.

In practice, increased electric goading is often observed at the entrance into the stunning chute as the handler has no other mean of dissolving a group of pigs and drive them individually through the stun race at a constant speed. In this study, climbing behavior was observed more often than turning around at this stage, as a result of the combined effect of electric goading and low space allowance. Indeed, it is known that when space is reduced, turning around is limited and pigs try to escape from the stressor (the electrical shock and the

- handler in this study) by climbing over the back of other group mates in search of protection
  within the group (Guise and Penny 1989; Lambooj and Engel 1991).

3 During lairage, similarly to Moss (1978) and Geverink et al. (1996), 70 to 90% of pigs 4 lay down after approximately an hour, which confirms the benefit of providing pigs with a 5 period of rest to allow them to recover from transport and the associated handling (Pérez et al. 6 2002; Warriss 2003). The increased level of activity and aggressiveness reported in pigs kept 7 in the larger group is consistent with the findings by Bryant and Ewbank (1972), Petherick 8 (1983) and Geverink et al. (1996), but contrasts with those of Andersen et al. (2004). 9 According to Turner et al. (1999), the behaviour of pigs kept in large groups compared to 10 small groups depends on their perception of the group size and on the availability of water 11 and food in the pen. If the space per pig and resources (food, water, etc.) are sufficient, it 12 appears that the level of activity and the frequency of agonistic acts do not increase. As all 13 pigs were fasting, the aggressiveness observed in this study, especially in the large groups, 14 might have been increased by the lack of food. Indeed, fasting often results in increased 15 fighting, especially in mixed pigs (Fernandez et al. 1995; Brown et al. 1999; Turgeon 2003).

16 Rough preslaughter handling and poor social environment in lairage may cause 17 psychological and physical stress in pigs as shown by the significant increase in blood 18 hormone levels, heart rate and body temperature reported in several studies (Faucitano 2001b; 19 Hemsworth et al. 2002). However, except for a slight effect of group size on cortisol, the 20 treatments applied had no effect on the levels of urinary hormones at slaughter in this study. 21 Overall, the urinary levels of CA recorded in this study were higher than those reported by 22 Mormède et al. (2004) and lower than those reported by Foury et al. (2005) for pigs 23 slaughtered in a commercial abattoir following transportation. On the other hand, cortisol 24 levels were lower than those reported by Mormède et al. (2004) and Foury et al. (2005). 25 These discrepancies between studies may be due to genetic differences (Hay and Mormède

1 1998; Mormède et al. 2004). Overall, the lack of strong differences between treatments 2 suggest that the pre-slaughter management applied in this study was stressful for all pigs and 3 may have masked treatment effects. For instance, the lairage environment was noisy (82 to 4 108 dB). Geverink et al. (1998) showed that the noise produced by the machinery, pressure 5 hoses, and pig and human vocalisations represents a source of stress with which pigs cope by 6 huddling and escaping from the source of sound. Talling at al. (1996) and Kanitz and 7 Tuchscherer (2005) also reported an increased blood cortisol level and heart rate in pigs 8 submitted to high sound intensity level (85-97 dB).

9 Although treatments affected the behaviour of pigs during handling and lairage, they 10 had no impact on stomach weight. This contrasts with the findings by Enck et al. (1989), that 11 gastric emptying was delayed in rats after stress. However, it is consistent with the 12 physiological response of pigs, which suggests that they were equally stressed, regardless of 13 treatment. In the present study, despite a fasting of 20 hours, 23.5% of stomachs were not 14 empty according to the criteria of Chevillon (1994). Of the stomachs that did not meet the 15 criteria, a high percentage contained liquid (79.4%). However, it should be noted that 16 treatments had no impact on stomach content, despite the fact that GH pigs drank more than 17 RH. The reason for the lack of impact of drinking behaviour during lairage on stomach 18 content weight and composition can be two-fold. On one hand, RH pigs might have drunk 19 more during the last two hours of lairage (not under observation), and compensated for their 20 lower drinking frequency in the first hour. On the other hand, given that liquid evacuation rate 21 from the stomach is higher than that of solid content (Gregory et al. 1990), the water 22 consumed by pigs during the first hour of lairage may have been absorbed or evacuated 23 before slaughter.

Given the precautions taken during the removal of the gastro-intestinal tract from the carcass to avoid content spillage at sampling, only one stomach was perforated during

1 evisceration. Hence, the treatments applied in this study did not appear to affect the efficacy 2 of the evisceration procedure. The lack of difference between treatments in microbial carcass 3 contamination and in the number of carcass trimmed for visual contamination and defects 4 suggest that the stress conditions applied in this study had limited impact on the carcass 5 microbial quality. Indeed, overall carcass contamination was very low in our experiment. The 6 levels of TAM, coliforms or E. coli counts measured on all carcasses were within the 7 guidelines of the Meat Hygiene Manual (CFIA 2006), independently of the treatment applied. 8 Carcass trimming occurred mainly on week 10 (16.3 % of the carcasses), for no obvious 9 reason. This suggests that carcass contamination at that time may have been caused by an 10 unexpected event independent of the preslaughter handling conditions under study. 11 Botteldoorn et al. (2003) showed a high variation in the incidence of contaminated carcasses 12 between different sampling days both at the same abattoir (between 3 and 52%) and between 13 abattoirs (between 0 and 70%).

The increased climbing behaviour provoked by electric prodding of pigs at unloading and right before slaughter increased the incidence of skin bruises on the carcass. Bruises may have been produced by the fore nails of pigs climbing onto the back of others while escaping from the source of stress (handler and electric goad) in a situation of high stocking density. This observation was already reported by Faucitano et al. (1998) in pigs lined up and handled with electric prods in a stunning chute not equipped with hold-down bars to prevent climbing activity.

The direct effect of electric prodding on the production of exudative pork is extensively reported in the literature (D'N Souza et al. 1998; Støier et al. 2001; Hemsworth et al. 2002; Hambrecht et al. 2005). Van der Wal et al. (1999) reported that only a very shortterm stress (1 min) immediately before stunning can be sufficient to produce exudative pork. Likewise, Hemsworth et al. (2002) reported that the nature of the interaction received by the

pigs (pats and strokes or slaps and electric goad hits) in the crowding pen prior to the final race leading to the stunning area was directly correlated with the fear of the handler. In the present study, the increased physical activity (climbing) a few minutes before slaughter caused by the presence of the handler in combination with the electric shock generated by the prod reduced muscle pH<sub>1</sub> and water-holding capacity of pork meat.

6 Despite the higher level of activity and aggressiveness observed in pigs kept in large 7 groups, group size had no effect on skin bruise score and had just a small, but not biologically 8 significant, effect on  $pH_{u}$ . These results are surprising as fighting in lairage is usually 9 associated with a high bruise score on the carcass and high muscle pHu due to the effects of 10 physical activity on glycogen levels at slaughter (Warriss 1996; Gispert et al. 2000). 11 Nevertheless, our results may support the findings by Andersen et al. (2004) that the 12 proportion of pigs not participating in fights was higher in the large group. According to Pagel 13 and Dawkins (1997), the probability of observing the same individuals fighting declines 14 sharply with increasing group size, because the energetic costs and physical injury associated 15 with establishing and maintaining a social hierarchy in a large group may encourage the 16 adoption of an alternative strategy. Thus, the fact that fewer animals may have been involved 17 in fights in the large group, might explain the lack of effect of increased agonistic acts on the 18 average skin bruise score and pork quality of the group.

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20

#### CONCLUSIONS

21

Poor handling increased the incidence of flight behaviours at unloading and aggressive behaviours along the lairage alleys and resulted in poorer pork quality. The level of activity and the frequency of aggression during lairage also increased with group size. However, group size had no impact on pork quality. The stressors applied on pigs in lairage did not

1	influence stomach weight at slaughter, suggesting that they did not contribute to stomach
2	emptying variation before slaughter. Furthermore, all samples (400 in total) were within the
3	guidelines of the Meat Hygiene Manual for the indicator organisms of the carcass hygienic
4	status (TAM, coliforms or E. coli counts), independently of the treatments applied (CFIA,
5	2006). Hence, no treatment tested had a negative effect on the hygiene level observed and all
6	carcasses were fit for commercialization. Further research is needed to study the effect of
7	drinking rate during lairage on stomach weight and content composition.
8	
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Variables	Unlo	ading		Stunning	Stunning raceway		
	RH	GH	Р	RH	GH	Р	
Climbing	$0.6 \pm 0.3$	0	*	$3.8 \pm 0.8$	$0.6 \pm 0.4$	*	
Turning around	$5.1 \pm 0.7$	$1.1 \pm 0.4$	***	$4.4 \pm 0.9$	$5.0 \pm 0.4$	NS	
Slipping	$1.3 \pm 0.3$	$0.1 \pm 0.1$	**	-	-		
<sup>z</sup> RH = rough handling;	GH = gentle h	andling					
$y_{-}$ = no measures							
	<u> </u>	NO	· C•				
*P < 0.05; **P < 0.01;	; *** <i>P</i> < 0.001	; INS = not si	gnificant	•			

 Table 2. Effect of handling quality (rough vs. gentle) and group size (10 pigs vs. 30 pigs)

Variables	H	Iandling	Z		Group size			
	RH	GH	SEM	Р	10	<b>30</b> <sup>y</sup>	SEM	P
Cortisol (ng/mg	14.82	13.29	0.79	NS	14.94	13.12	0.79	*
creatinine)								
Cortisone (ng/mg	23.66	21.66	1.27	NS	23.29	21.49	1.23	NS
creatinine)								
Noradrenaline (ng/mg	16.02	15.52	0.58	NS	14.76	16.84	0.65	NS
creatinine)								
Adrenaline (ng/mg	8.26	8.11	0.35	NS	7.70	8.70	0.36	NS
creatinine)								
Dopamine (ng/mg	13.17	12.83	0.59	NS	13.14	12.86	0.63	NS
creatinine)								
RH = rough handling; GH =	= gentle ha	ndling						
Observation made on a sub	sample of	10 pigs v	vithin ea	ch gro	up of 30			
P < 0.05; NS = not signific	-	10		U	I			
F < 0.05, $NS = 100$ signific	allt							

on urinary hormone levels at slaughter

## Table 3. Effect of handling quality (rough vs. gentle) and group size (10 pigs vs. 30 pigs) on

stomach weights at slaughter

Variables	]	Handling <sup>z</sup>			Group size			
	RH	GH	SEM	Р	10	<b>30</b> <sup>y</sup>	SEM	Р
Full stomach (g)	817.4	810.5	18.3	NS	797.4	830.5	19.7	NS
Emptied stomach (g)	413.1	413.3	4.1	NS	412.1	414.2	4.2	NS
Stomach content (g)	381.7	376.1	15.6	NS	363.4	394.5	16.9	NS
$\mathbf{DM}(\%)^{\mathbf{x}}$	14.5	13.9	0.4	NS	14.3	14.0	0.4	NS

- $3 \quad ^{x}DM = dry matter$
- 4 NS = not significant

 Table 4. Effect of handling quality (rough vs. gentle) and group size (10 pigs vs. 30 pigs)

RHGHLiquid142148Feed3532Sawdust8141 ${}^{z}RH = rough handling; GH = gentle handling}$ 2 ${}^{y}Observation made on a subsample of 10 pigs with$	10 150 29 7 in each group of 30	<b>30</b> <sup>y</sup> 140 38 15
Feed3532Sawdust8141 ${}^{z}RH = rough handling; GH = gentle handling$	29 7	38
Sawdust8141 ${}^{z}RH = rough handling; GH = gentle handling$	7	
$^{z}$ RH = rough handling; GH = gentle handling		15
	in each group of 30	
$2^{y}$ Observation made on a subsample of 10 pigs with	in each group of 30	
2 Observation made on a subsample of 10 pigs with		
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L Contraction of the second		
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on number of stomachs containing liquid, sawdust or feed

### Table 5. Effect of handling quality (rough vs. gentle) and group size (10 pigs vs. 30

				Statististics						
			$\overline{x}^{\mathbf{x}}$	<b>SD</b> <sup>w</sup>	Log A <sup>v</sup>	N <sup>u</sup>	% <sup>t</sup>			
	Handling <sup>z</sup>	RH	1.73	0.32	1.85	4.16	100			
TAM		GH	1.69	0.32	1.81	4.10	100			
	Group size	10	1.73	0.33	1.85	4.16	100			
		30 <sup>y</sup>	1.69	0.32	1.81	4.11	100			
	Handling	RH	1.68	0.69	2.23	5.20	79			
Coliforms		GH	1.71	0.64	2.18	4.96	78			
	Group size	10	1.73	0.67	2.24	5.19	82			
		30 <sup>y</sup>	1.66	0.66	2.16	4.97	75			
	Handling	RH	1.45	0.69	1.99	5.09	52			
		GH	1.49	0.57	1.86	4.34	68			
E. coli	Group size	10	1.48	0.58	1.87	4.77	65			
		<b>30</b> <sup>y</sup>	1.46	0.67	1.98	4.94	55			

### pigs) on carcass contamination : TAM, coliforms and E. coli counts

1  $^{z}RH = rough handling; GH = gentle handling$ 

2 <sup>y</sup> Observation made on a subsample of 10 pigs within each group of 30

3 <sup>x</sup> Mean of Log<sub>10</sub> CFU/cm<sup>2</sup> for TAM, mean of Log<sub>10</sub> CFU/983cm<sup>2</sup> for coliforms and *E. coli* counts

4 <sup>w</sup> Standard deviation of the log<sub>10</sub> counts

5  $^{v}$  Log<sub>10</sub> of the arithmetic mean

6 <sup>u</sup> Log<sub>10</sub> of the total number recovered from 10 x 983 cm<sup>2</sup>

7 <sup>t</sup>Percentage of samples with countable colonies on the grids

8

 Table 6. Effect of handling quality (rough vs. gentle) and group size (10 pigs vs. 30 pigs)
 on meat quality traits

Variables	Handling <sup>z</sup>			Group size				
	RH	GH	SEM	P	10	<b>30</b> <sup>y</sup>	SEM	Р
Skin bruise score <sup>x</sup>	1.53	1.36	0.06	0.06	1.46	1.43	0.06	NS
pH <sub>1</sub>	6.01	6.11	0.05	**	6.05	6.06	0.05	NS
рНи	5.60	5.58	0.02	NS	5.58	5.61	0.02	**
EC <sup>w</sup> (μS)	5.84	4.97	0.22	**	5.45	5.36	0.22	NS
Subjective colour score <sup>v</sup>	2.45	2.42	0.05	NS	2.42	2.44	0.05	NS
L*	52.44	52.17	0.28	NS	52.26	52.36	0.28	NS
a*	8.72	8.44	0.13	*	8.64	8.52	0.13	NS
b*	6.47	6.34	0.15	NS	6.45	6.35	0.15	NS
Drip loss (%)	7.58	6.96	0.29	*	7.38	7.15	0.29	NS

1  $^{z}RH$  = rough handling; GH = gentle handling

2 <sup>y</sup> Observation made on a subsample of 10 pigs within each group of 30

3 <sup>x</sup> According to photographic standards (from 1 =none to 5 = severe; MLC 1985)

4 <sup>w</sup>EC = electrical conductivity measured by Pork Quality Meter (Intek, Aichach, Germany; >

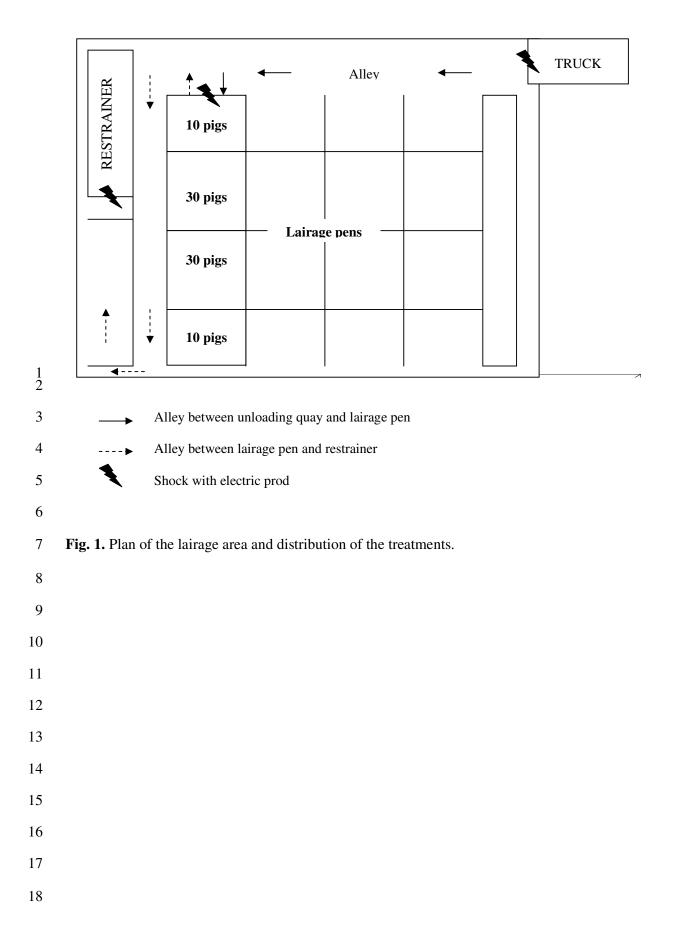
5 5  $\mu$ s = fast post mortem glycolysis leading to unacceptable pork; Barton-Gade et al. 1996)

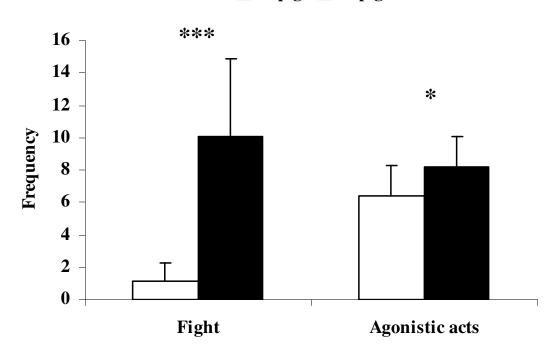
6 <sup>v</sup> According to Japanese Color Scales (from 1 = pale to 6 = dark; Nakai et al. 1975)

7 \**P* < 0.05; \*\**P* < 0.01; NS = not significant

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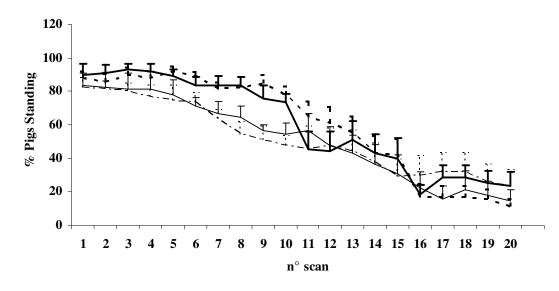


🗆 10 pigs 🔳 30 pigs



Fig. 2. Frequency of fighting and agonistic acts (bites and head knocks) during lairage according to
group size (10 pigs vs. 30 pigs; mean ± SD; \*P < 0.05; \*\*\*P < 0.001). Observation made by scan</li>
sampling on a subsample of 10 pigs within each group of 30.





— Gentle, 10 – – – Rough, 10 — – Gentle, 30 – – – Rough, 30

Fig. 3. Percentage of pigs standing during lairage according to handling quality (gentle vs. rough) and
group size (10 pigs vs. 30 pigs; mean ± SD). Observation made by scan sampling on a subsample
of 10 pigs within each group of 30 pigs.