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Reduced contamination of pig carcasses using an alternative pluck set removal procedure during slaughter

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ABSTRACT

This study compared the current pig slaughter procedure where the pluck set is completely removed with a procedure where the pluck set is partially removed, leaving the highly contaminated oral cavity, tonsils and tongue untouched. The effect on carcass contamination was investigated by enumerating hygiene indicator bacteria (total aerobic count, *Enterobacteriaceae* and *E. coli*) and cefotaxime-resistant *E. coli* (CREC) as well as assessing *Salmonella* and *Yersinia enterocolitica* presence on the sternum, elbow and throat of pig carcasses. Using the alternative pluck set removal, significantly lower mean numbers of hygiene indicator bacteria on throat samples and *E. coli* on elbow samples were found. Less pig carcasses were highly contaminated and a lower presence and level of CREC was observed. No difference in *Salmonella* or *Yersinia enterocolitica* presence was seen. The data in this study can help to assess the effect of this alternative procedure on the safety of pork and subsequently public health.

1. Introduction

The removal of the pluck set during pig slaughter has been designated as an important source for pig carcass contamination (Hald, Wingstrand, Swanenburg, von Altrock, & Thorberg, 2003). The pluck set (i.e. the liver, lungs, heart, trachea, esophagus, and tongue) is removed from the pig carcass after evisceration and opening of the oral cavity but before splitting of the carcass (Swart, Evers, Simons, & Swanenburg, 2016). The oral cavity, with the tongue and palatine tonsils, is known to harbor a high bacterial load, including human pathogenic bacteria such as *Salmonella* and *Yersinia enterocolitica,* as well as antibiotic resistant bacteria (Borch, Nesbakken, & Christensen, 1996; Van Damme et al., 2017). Furthermore, intranasal and oral inoculation of *Salmonella* and human pathogenic *Y. enterocolitica*, respectively, showed that both pathogens colonize the tonsils more rapidly compared to the colon and remain present for a longer time period in the tonsils than in the colon, which may indicate that tonsils are more important as reservoir for both pathogens than the colon (Fedorka-Cray, Kelley, Stabel, Gray, & Laufer, 1995; Thibodeau, Frost, Chenier, & Quessy, 1999).

The prevalence of human pathogenic *Yersinia enterocolitica* in pig tonsils at slaughter has been described in various studies throughout the EU, with values varying between 11% and 88% (Bonardi et al., 2013; de Boer & Nouws, 1991; Fredriksson-Ahomaa, Bucher, Hank, Stolle, & Korkeala, 2001; Fredriksson-Ahomaa, Stolle, & Stephan, 2007; Gurtler, Alter, Kasimir, Linnebur, & Fehlhaber, 2005; Korte, Fredriksson-Ahomaa, Niskanen, & Korkeala, 2004; Thibodeau et al., 1999). Similarly, *Salmonella* has been found in 9.9% of pig tonsils in Portugal (Vieira-Pinto, Temudo, & Martins, 2005). Human pathogenic *Y. enterocolitica* and *Salmonella* have also been found on pig tongues at slaughter with a prevalence of 75% and 7.9%, respectively (Fredriksson-Ahomaa et al., 2001; Hald, Wingstrand, Swanenburg, von Altrock, & Limpitakis, 1999). In Belgium, particularly, three studies reported the prevalence of human pathogenic *Y. enterocolitica* in pig tonsils, ranging from 28% to 55%, and enumeration data describing numbers up to 6.0 $\log_{10}$ CFU/g tonsil with mean levels between 4.0 and 4.5 $\log_{10}$ CFU/g tonsil (Van Damme et al., 2015; Van Damme, Habib, & De Zutter, 2010; Vanantwerpen, Van Damme, De Zutter, & Houf, 2014). *Salmonella* was found in 18% of the tonsils of pigs at slaughter, with numbers up to 5.0 $\log_{10}$ CFU/g tonsil (Van Damme, Mattheus, Betrand, & De Zutter, 2018). Findings of Van Damme et al. (2018) and De Busser et al. (2011) also proved the presence of *Salmonella* in oral cavities of

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pigs at slaughter with 54% and 14% of the oral cavities being positive after polishing and bleeding, respectively, and numbers exceeding 4.0 $\log_{10}$ CFU/cm^2.

Regarding the presence of general hygiene indicator bacteria, Van Damme et al. (2018) reported mean levels for total aerobic bacteria, *Enterobacteriaceae* and *E. coli* of 6.63, 5.75, and 5.56 $\log_{10}$ CFU/g in tonsils of pigs at slaughter in Belgium, respectively. In a study by Fredriksson-Ahomaa, Gerhardt, and Stolle (2009), executed in Germany, mean values of 7.12 and 5.52 $\log_{10}$ CFU/g tonsil for total aerobic bacteria and *E. coli*, respectively, were found. Gill and Jones (1998) enumerated *E. coli* on pig tongues in Canada, which resulted in mean numbers of 2.3 $\log_{10}$ CFU/tongue swab. Counts of the total aerobic bacteria, *Enterobacteriaceae* and *E. coli* in oral cavities resulted in mean numbers of 5.5, 2.6 and 2.3 $\log_{10}$ CFU/cm^2, respectively (Van Damme et al., 2018). Cefotaxime-resistant *E. coli* have been detected in pig tonsils as well, in numbers varying between 1.0 and 5.6 $\log_{10}$ CFU/g tonsil in 47% of the pigs at slaughter in Belgium (Van Damme et al., 2017).

Given the high presence of hygiene indicator bacteria and well-known pathogenic bacteria, the pluck set removal has been determined to be a critical control point during pig slaughter for carcass contamination (Borch et al., 1996; Kapperud, 1991). Both based on epidemiological and molecular *Y. enterocolitica* studies, tonsils have been shown to directly contaminate other parts of the pluck set (e.g. liver, lungs and heart) and the rest of the pig carcass (Fredriksson-Ahomaa, Korte, & Korkeala, 2000; Laukkanen et al., 2009; Van Damme et al., 2015; Vilar, Virtanen, Laukkanen-Ninios, & Korkeala, 2015). Besides direct contamination from the oral cavity, tongue or tonsils to other pluck set parts and the pig carcass, bacteria may also spread to the environment, such as the pluck set hook, which can eventually lead to cross-contamination (Fredriksson-Ahomaa et al., 2000). A study by Bonardi et al. (2013) even concluded that carcass contamination by *Salmonella* and *Y. enterocolitica* from the tonsils is more likely to be attributed to cross-contamination than to self-contamination.

Consequently, it has been suggested to adapt the procedure for the removal of the pluck set during pig slaughter in order to avoid contamination of the carcass, pluck set, environment and subsequently cross-contamination (Borch et al., 1996; Fredriksson-Ahomaa et al., 2000; Kapperud, 1991; Laukkanen et al., 2009; Vilar et al., 2015). While an alternative removal of the pluck set without opening the oral cavity and leaving the tongue and tonsils inside the unopened head is frequently used in France (Denis, Minveille, Feurer, Desmonts, & Carneil, 2012) and some studies have briefly investigated this slaughter adaptation (Christensen & Luthje, 1994; Olsen, Jensen, Dahl, & Christensen, 2001), there is a lack of data about the all-over effect of such an alternative pluck set removal on the contamination of pig carcasses. Furthermore, official meat inspection in slaughterhouses in Europe demands the visual inspection of the fauces, tongue and throat of every pig carcass before it leaves the slaughter line (Commission Regulation (EU) No 219/2014), which involves opening of the oral cavity. Therefore, the aim of this study was to comprehensively investigate the effect of an alternative pluck set removal on pig carcass contamination regarding numbers of hygiene indicator bacteria (total aerobic count, *Enterobacteriaceae* and *E. coli*) and cefotaxime-resistant *E. coli* as well as the presence of *Salmonella* and human pathogenic *Y. enterocolitica*. This, in order to properly inform the industry about the effect involved in such a slaughter adaptation and to be able to estimate the potential effect on the quality and safety of pork. Finally, this study could help to correctly advise legal authorities regarding adaptations of the current meat inspection depending on the public health benefits of this method.

2. Material and methods

2.1. Slaughter procedures

In this study, the standard pluck set removal during pig slaughter was compared to an alternative pluck set removal. During the standard removal procedure, the liver, lungs, heart, trachea, esophagus, tongue, pharynx and tonsils are taken out of the carcass, all at once. In the alternative method, the pluck set was only partly removed from the carcass. Therefore, the trachea and esophagus were cut which made it possible to only take out the lungs, liver and heart from the carcass. Meanwhile, the oral cavity remained closed with the tongue, tonsils, pharynx and remaining parts of the trachea and esophagus inside. These parts were only removed after the trimming and first fast-cooling of the pig carcass, at the point where the head was detached from carcass and deboned.

2.2. Sampling

Two Belgian slaughterhouses with a slaughter speed of 600 pigs and 300 pigs per hour, respectively, were visited 6 times each, between February 2016 and July 2016. During every visit, between 2 and 3 h after the start of the slaughter activities, one slaughter batch of at least 200 fattening pigs was selected. Half of the pigs of the selected batch were slaughtered with the standard pluck set removal and the other half slaughtered using the alternative pluck set removal. For each slaughter method, every third pig passing through the slaughter line was sampled until ten pig carcasses per method were sampled, in total. Accordingly, 12 batches were investigated and 20 pig carcasses per batch were sampled (10 alternatively and 10 normally slaughtered) which resulted in a total of 240 pig carcasses (120 alternatively and 120 normally slaughtered). Samples were taken immediately after removing the pluck set and splitting the carcass by using cellulose sponge swabs (3 M, Diegem, Belgium) that were soaked in 25 mL Buffered Peptone Water (BPW; Bio-Rad Laboratories, Marnes-La-Coquette, France). From each carcass, three areas were separately swabbed, being the elbow, throat and sternum (approximately 100 cm^2 each). For each pig, all samples were taken from the same carcass half (either the left or the right carcass half), while alternating between the left and right carcass half among the different carcasses (e.g. for first carcass the three areas were sampled from the left carcass half, and for the second carcass all samples were taken from the right half). Next, all samples were transported to the lab under chilled conditions (below 7 °C) and processed for analysis the same day. Due to operator errors, two elbow samples (originating from two pig carcasses of two different batches, alternatively slaughtered in the same slaughterhouse) could not be taken and processed.

2.3. Microbiological analyses

2.3.1. Hygiene indicator bacteria

Samples were subjected to a one-minute homogenization in a stomacher (Colworth Stomacher 400, Steward Ltd., London, UK) prior to analysis. Subsequently, based on Van Damme et al. (2018), 100 µL of this initial suspension was inoculated directly on Plate Count Agar (PCA; Bio-Rad Laboratories, Marnes-La-Coquette, France), Rapid *Enterobacteriaceae* (RAPIDEN, Bio-Rad Laboratories, Marnes-La-Coquette, France) and Tryptone Bile Glucuronic agar (TBX, Bio-Rad Laboratories, Marnes-La-Coquette, France) using a spiral plate machine (Eddie Jet, IUL Instruments, Barcelona, Spain) to assess the number of total aerobic bacteria, *Enterobacteriaceae* and *E. coli*, respectively (detection limit of 0.40 $\log_{10}$ CFU/cm^2). PCA plates were incubated for 48 h at 30 °C, RAPIDEN plates for 24 h at 37 °C and TBX plates for 24 h at 44 °C, after which colonies were enumerated.

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