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Characterization of the *Arcobacter* contamination on Belgian pork carcasses and raw retail pork

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Abstract

In the present study, the occurrence of *Arcobacter* was assessed at four sites on 169 porcine carcasses (foreleg, chest, pelvis and ham) at different stages of slaughter and 47 pork products at retail. Carcass swab samples were enriched in *Arcobacter* broth containing 5-fluorouracil, amphotericine B, cefoperazone, novobiocine and trimethoprim as selective supplement. After microaerobic incubation, arcobacters were isolated using *Arcobacter* selective agar plates, containing the selective supplement described above. Some carcass samples and all pork samples were also examined quantitatively. All 862 isolates were identified by a species-specific m-PCR-assay and 182 isolates were further characterized by ERIC-PCR. Arcobacters were isolated from one or more sampling places on 96.4% of the carcasses, with the foreleg and the chest area as the two most contaminated sites. Furthermore, *A. cryaerophilus* was the most common species. Chilling decreased the number of positive carcasses, but did not eliminate all arcobacters. Direct isolation revealed that only a few carcasses were contaminated with arcobacters on foreleg and/or chest at levels higher than 10^2 cfu/100 cm^2. Characterization demonstrated a large heterogeneity among the isolates, with ten genotypes present on more then one site per carcass. Fourteen genotypes were simultaneously present on carcasses from different herds slaughtered on the same day, which may indicate cross-contamination. Arcobacters were present in 21% of the pork samples taken at retail, but contamination levels did not exceed 100 cfu per gram. Characterization of the *A. butzleri* and *A. cryaerophilus* isolates indicated an additional contamination during processing at retail.

Keywords: *Arcobacter*; Porcine carcasses; Minced pork; Pork chops

1. Introduction

The genus *Arcobacter* encompasses Gram-negative slender curved bacteria formerly known as aerotolerant campylobacters (Vandamme and De Ley, 1991). Arcobacters differ from the closely related campylobacters by their ability to grow at lower temperatures and in air (Vandamme et al., 1991). Since the creation of *Arcobacter* as a second genus within the family Campylobacteraceae (Vandamme and De Ley, 1991) six species have been characterized. Besides the two environmental-related species *Arcobacter nitrofigilis* and *Arcobacter halophilus*, for which no association with humans or animals has yet been reported, current research has focused on *Arcobacter butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. cibarius* since those species are considered as emerging foodborne pathogens (McClung et al., 1983; Vandamme et al., 1991; Donachie et al., 2005; Houf et al., 2005). In humans, predominantly *A. butzleri* has been associated with enteritis and occasionally septicemia, though also *A. cryaerophilus* and recently *A. skirrowii* have been isolated from stool of diarrheic patients (Vandenberg et al., 2004; Wybo et al., 2004). Similar clinical features to *Campylobacter jejuni* infection are displayed, but a higher association with persistent and watery diarrhea has been reported (Vandenberg et al., 2004).

Arcobacters are commonly present on food of animal origin with the highest prevalence for poultry, followed by pork and beef (Rivas et al., 2004). Though the exact contribution of food of animal origin to human infection remains to be determined, handling raw meat, cross-contamination and the consumption of undercooked meat products are the probable routes of infection (Lehner et al., 2005).

Although arcobacters were isolated from the organs of porcine fetuses as long ago as 1977, their clinical role has hardly

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been investigated (Ellis et al., 1977, 1978; On et al., 2002). In more recent studies, arcobacters have been isolated from uterine and oviductal tissues of sows with reproduction disorders (de Oliveira et al., 1997), and from infertile sows with vaginal discharge (Anonymous, 1998). However, they have also been isolated from vaginal swabs of healthy and normal reproducing sows (On et al., 2002; Kabeya et al., 2003). In boars, arcobacters have been recovered from preputial swabs, but they were not detected in the semen (de Oliveira et al., 1999). Besides the association with reproduction problems, one study has reported the presence of arcobacters in the stomachs of pigs with gastric ulcers (Suarez et al., 1997).

Arcobacters have also been isolated from feces of apparently healthy porkers of different ages (Hume et al., 2001; Kabeya et al., 2003; Van Driessche et al., 2003; 2004; Ho et al., 2006). In recent Belgian studies, arcobacters have been isolated from porcine feces in levels up to 10^4 cfu/g, with *A. butzleri* as the most common species, although co-colonization with two or three species was sometimes found (Van Driessche et al., 2003, 2004). A wide heterogeneity has been detected among the *Arcobacter* isolates from pigs on farm level (Hume et al., 2001; Van Driessche et al., 2004). Recently, an intra-uterine transmission route from sows to piglets and a postnatal infection route from pigs or environment to piglets has been suggested (Ho et al., 2006).

Since their isolation from the feces of porkers at the slaughterhouse, it has been suggested that contamination of pork products probably occurs by fecal contamination of the carcasses during slaughter (Ohlendorf and Murano, 2002; Van Driessche et al., 2004).

To date, only a limited number of studies report the occurrence of *Arcobacter* on pork, and no information is available about carcass contamination. Furthermore, as no standard *Arcobacter* isolation method is presently set down, comparison of data remains difficult. In the present study the occurrence of *Arcobacter* was assessed by a prevsiously developed *Arcobacter* selective method on carcasses at different stages of slaughter and two types of pork retail products. In both cases, the isolates were further characterized in order to obtain insight in the heterogeneity of the *Arcobacter* population present.

2. Materials and methods

2.1. Occurrence of Arcobacter on pork carcasses at slaughterhouse

From October to December 2004, sampling was done on nine occasions in a Belgian pork slaughterhouse with a processing capacity of approximately 300 pigs per hour. The slaughter process is described in brief below. The animals were first stunned using 80% CO_2 and secured to an overhead conveyor rail by a chain looped around one of the hind legs. The stunned animals were immediately stuck by cutting the main blood vessel in the neck using a hollow knife and then bled over trough a trench. Each animal was scalded for 4 min (at 63 °C) and immediately mechanically dehaired. Pigs were secured to the overhead conveyor by hooks behind the Achilles' tendons of both legs after which they were dry polished. Carcasses were singed by gas burners and thereafter they were given a pre-evisceration wash with potable warm water. Evisceration was performed by first detaching and bagging the anus and rectum followed by splitting open the belly and cutting the connective tissues joining the viscera to the carcass. The diaphragm, heart, lungs, trachea and tongue were manually removed along with the digestive tract. The carcass was subsequently cut along the midline using an automatic splitting saw and heads were removed. Finally, carcasses were first shock-chilled for 75 min by forced ventilation and then stored in cooling rooms at 3 °C for at least 24 h.

In total, 169 carcasses were sampled of which 18 on the slaughter line immediately after evisceration, 80 were sampled after 6 h in the cold store, and 71 were sampled 24 h after evisceration when the carcasses had reached a core temperature of 7 °C (Table 1). Each carcass was sampled only once. Within the samples of a same processing time, different farms were included.

Of each carcass, samples were taken on four different places, according to the EU Decision 2001/471/EC of 8 June 2001 (Fig. 1). A 100 cm^2 area of the ham, pelvis and foreleg and a 300 cm^2 area of the chest (sternum region) was sampled using a cotton swab moistened in buffered peptone water. Swabs were placed into sterile stomacher bags and transported chilled to the lab.

Arcobacters were isolated according to the method developed by Houf et al. (2001). The swabs were homogenized with 15 ml *Arcobacter* broth (containing 24 g l^{-1} *Arcobacter* broth (CM 965, Oxoid, Basingstoke, UK), 100 mg l^{-1} 5-fluorouracil (F-6627, Sigma, St. Louis, USA), 10 mg l^{-1} amphotericin B (A-4888, Sigma), 16 mg l^{-1} cefoperazone (C-4292, Sigma), 32 mg l^{-1} novobiocin (N-1628, Sigma), 64 mg l^{-1} trimethoprim (T-0667, Sigma) and 50 ml l^{-1} lyzed defibrinated horse blood (E and O Laboratories Ltd., Bonnybridge, Scotland) using a stomacher blender (Eddy Yet, IUL Instruments, Barcelona, Spain) for 1 min at normal speed. The homogenates were incubated for 48 h at 28 °C under microaerobic conditions by evacuating 80% of the normal atmosphere and introducing a gas mixture of 8% CO_2, 8% H_2 and 84% N_2 into the jar. This incubation atmosphere was used for all further *Arcobacter* isolations. After incubation, 50 µl of each homogenate was streaked onto an *Arcobacter* agar plate

Table 1
Occurrence of arcobacters on pork carcasses at different processing times

Sampling place	Sampling time		
	Directly post evisceration ($n=18$)	6 h post evisceration ($n=80$)	24 h post evisceration ($n=71$)
Foreleg	18 (100%)	74 (92.5%)	52 (73.2%)
Chest	17 (92.4%)	75 (93.4%)	56 (78.9%)
Pelvis	17 (92.4%)	47 (58.8%)	13 (18.3%)
Ham	15 (83.3%)	57 (71.3%)	22 (31.0%)
Number of carcasses contaminated at least one area	18	80	65