Spatial Distribution of the Emerging Foodborne Pathogen *Arcobacter* in the Gastrointestinal Tract of Pigs

Sarah De Smet, Lieven De Zutter, and Kurt Houf

**Abstract**

Pigs are important reservoirs for *Arcobacter*. Since 1978, *Arcobacter* species have been associated with reproduction disorders, but excretion by clinically healthy pigs has been frequently reported as well. Information on *Arcobacter* colonization of the porcine gastrointestinal tract is lacking. In the present study, gastrointestinal tracts of 12 pigs were collected, and the content and mucus of eight sections were examined. *Arcobacters* were enumerated and isolated by a selective quantitative and qualitative method, respectively, and identified by multiplex-polymerase chain reaction (PCR). Their genetic diversity was examined by enterobacterial repetitive intergenic consensus PCR and pulsed-field gel electrophoresis.

*Arcobacter* species were isolated from at least two gastrointestinal sections of all pigs in levels up to $10^5$ colony-forming units (CFU) g$^{-1}$ in content and $10^4$ CFU g$^{-1}$ in mucus. Characterization of the isolates revealed a high degree of genotypic diversity. In general, the highest counts, and greatest species and strain diversity was obtained from the large intestine, and especially from the rectum. Though *Arcobacter* strains were mostly detected in one gastrointestinal section, several unique strains were also recovered from the content and/or mucus of various gastrointestinal sections of individual pigs. In the gastrointestinal tract, *Arcobacter* is present with species distributions, numbers, and strain heterogeneity comparable to those reported on porcine carcasses post slaughter, thus confirming the potential route of transmission to carcasses by fecal contamination during processing.

**Introduction**

Since the creation in 1991 of the genus *Arcobacter* as a second genus within the family *Campyllobacteraeae*, 13 species have been characterized (De Smet et al., 2011a; Figueras et al., 2011b; Houf et al., 2009; Vandamme et al., 1991). At present, six species are associated with humans and animals, whereas the others seem to be more environmentally related (Collado et al., 2011; Figueras et al., 2011a; Houf et al., 2009).

In humans, predominantly *Arcobacter butzleri* has been isolated from patients with enteritis and occasionally septicemia (Houf and Stephan, 2007). Infection probably occurs through the consumption of contaminated drinking water and food, in particular, poultry products, pork, and beef (De Smet et al., 2010; Ho et al., 2006a; Jacob et al., 1993; Van Driessche and Houf, 2007a). Other risk factors are contact with pets and person-to-person contact (Fera et al., 2009; Houf et al., 2008; Vandamme et al., 1992a).

Though the first reports of *Arcobacter* in farm animals described diseases such as reproduction disorders, mastitis, and enteritis (Ellis et al., 1978; Nell et al., 1982; Vandamme et al., 1992b), *Arcobacter* has by now been isolated from the feces of healthy farm animals all over the world (Van Driessche et al., 2003; Wesley et al., 2000).

Previous research has shown that pigs are an important *Arcobacter* reservoir (Ho et al., 2006b; Van Driessche et al., 2004), in contrast to broilers, from whose intestinal content *Arcobacter* species have rarely been recovered (Ho et al., 2008; Van Driessche and Houf, 2007b). Transfer of fecal material onto carcasses during slaughter processing is accepted as the major source for porcine carcass contamination (Van Driessche and Houf, 2007b). As with other foodborne pathogens, the gastrointestinal tract of the animals is also considered a preferential site for colonization and eventually excretion for *Arcobacter*. However, no information is currently available on the *Arcobacter* presence, or species and strain distribution within the porcine gastrointestinal tract. Therefore, the present study aims to determine the numbers, and species and strain diversity of *Arcobacter* in the different sections of the gastrointestinal tract of pigs at slaughter age.

**Materials and Methods**

**Sampling of gastrointestinal content and mucus**

In this study, gastrointestinal tracts of a total of 12 unrelated pigs (hereafter identified as pigs 1–12, with mean weight of approximately 110 kg) were collected immediately...
post-mortem veterinary inspection on four occasions between March and October 2011 from two Belgian slaughterhouses. After both the esophagus and the terminal end of the rectum were tied off with plastic clips, the gastrointestinal tracts were individually packed in plastic bags, transported under cooled conditions, and processed within 2 h. In the laboratory, the stomach, and different sections of the small intestine (duodenum, jejunum, and ileum) and large intestine (caecum, colon ascendens, colon descendens, and rectum) were tied off and then cut out. The surface contamination was eliminated by immersing the sections in ethanol for 10 s, as previously described in Van Driessche and Houf (2007b). After evaporation of the ethanol in air, each section was opened with sterile utensils and a minimum of 1 g of the content was 1/10 diluted in Arcobacter-selective enrichment broth (Van Driessche et al., 2003). The broths were homogenized in a stomacher blender (IUL Instruments, Barcelona, Spain) (Van Driessche et al., 2003). In addition to the gastrointestinal content, mucus from each gastrointestinal section was collected from pigs 11 and 12. For this, the gastrointestinal sections were opened, and the content was sampled and then removed. To remove the remaining content, the sections were gently rinsed with phosphate-buffered saline (PBS), and the mucus was then scraped from the mucosa with a scalpel. The mucus samples were further processed as described above for the content samples.

**Enumeration and isolation of Arcobacter**

To determine the number of arcobacters within each section, 100 μL of each homogenate was directly inoculated in duplicate onto an Arcobacter-selective agar plate by spiral plating (Eddy Jet, IUL Instruments, Barcelona, Spain), as described previously by Van Driessche et al. (2003). The agar plates were incubated under microaerobic conditions for 48 h at 28°C in a jar from which 80% of the normal atmosphere had been evacuated and a gas mixture of 8% CO₂, 8% H₂, and 84% N₂ introduced. The plates were examined with a stereomicroscope with Henry transillumination, and the typically bluish colonies were counted (Houf and Stephan, 2007). A maximum of 30 colonies per gastrointestinal section per pig were randomly picked, subcultured onto blood agar plates, and incubated as described above.

To detect levels of Arcobacter below 10² colony-forming units (CFU) g⁻¹, a selective enrichment was performed by incubating all broths at 28°C for 48 h. Then, 100 μL of each broth was streaked onto an Arcobacter-selective agar plate and incubated as described above. Bacterial growth was checked every 24 h up to 72 h. One typical colony per agar plate was subcultured onto a blood agar plate.

The bacterial growth from the blood agar plates was stored in cryovials (Microbank; Pro-Lab Diagnostics, Richmond Hill, ON, Canada) at −80°C for later species identification and characterization.

**Identification and characterization at strain level**

Each isolate was cultivated again on a blood agar plate, and a 0.5-mL cell suspension was diluted in PBS to prepare the template DNA. Genomic DNA was extracted by the guanidine thiocyanate method (Pitcher et al., 1989). The DNA concentration was determined spectrophotometrically (BioPhotometer, Eppendorf, Hamburg, Germany) at 260 nm and adjusted to a concentration of about 50 ng μL⁻¹. Two microliters were used as a DNA template in the Arcobacter species-specific multiplex–polymerase chain reaction (PCR) assay of Douidah et al. (2010). Amplified products were size separated by agarose gel electrophoresis in 1% agarose Tris-Borate-EDTA gels at 100 V for 90 min.

A maximum of 12 colonies per Arcobacter species per gastrointestinal section per pig were randomly selected, and strains were characterized by enterobacterial repetitive interspecific consensus (ERIC)–PCR (Houf et al., 2002). The resulting banding patterns were used to evaluate the strain diversity comprising DNA fragments between 100 and 2072 bp. Computer-based normalization and interpolation of the DNA profiles and numerical analysis, using the Pearson product moment correlation coefficient with 1% position tolerance, were performed using the GelCompar 4.2 software package (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were constructed using the unweighted pair group linkage analysis method (UPGMA). For convenience, the correlation level was expressed as percentage similarity. As in previous studies, DNA patterns that differed by one or more DNA fragments were considered to be different genotypes (Aydin et al., 2007; Houf et al., 2003).

After ERIC-PCR analysis, genotypes that differed a maximum of two fragments and presented in more than one gastrointestinal section were subsequently characterized by pulsed-field gel electrophoresis (PFGE). The Arcobacter protocol by Son et al. (2006) was applied with KpnI (Fermentas GmbH, St. Leon-Roth, Germany) as the restriction enzyme. The Salmonella Braenderup strain H9812, restricted with XhoI (Invitrogen, Paisley, UK), was used as molecular size standard. The PFGE patterns were analyzed using the GelCompar 4.2 software program, and clusters were constructed using the Dice coefficient and UPGMA. Patterns that differed in two or more fragments were considered to be different strains (Oporto et al., 2007). In addition, isolates showing similar KpnI-PFGE patterns were further analyzed in a second PFGE with the restriction enzyme NruI (Fermentas GmbH, Sankt Leon-Rot, Germany) using the PulseNet (Smal) conditions for Campylobacter jejuni (Ribot et al., 2001).

**Results**

All pigs were clinically healthy according to ante- and post-mortem inspections by veterinarians, and content was present in each section of their gastrointestinal tract. Arcobacter spp. were isolated from two or more sections per pig. Numbers and species distribution are presented in Table 1. Arcobacter was isolated from the content of all sections examined, ranging from five pigs (duodenal and jejunal content) to 11 of the 12 (rectal content; Table 1).

In general, the highest Arcobacter numbers were present in the large intestine (except for pig 11). Except for two pigs (pigs 4 and 8), the highest Arcobacter numbers were counted in the rectal content. Ten pigs had, in certain sections, more than 100 Arcobacter colonies per gram, whereas in pigs 1 and 5, Arcobacter was only detected after selective enrichment of the samples. The Arcobacter counts in the different gastrointestinal sections of the 12 pigs are also presented in Table 1. Arcobacter species were not always isolated from the mucus of one gastrointestinal section, although they were isolated from the corresponding content, and vice versa.
Table 1. *Arcobacter* Load and Species Distribution in the Different Sections of the Gastrointestinal Tract and in Mucus

<table>
<thead>
<tr>
<th>Pig</th>
<th>Stomach</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Caecum</th>
<th>Colon ascendens</th>
<th>Colon descendens</th>
<th>Rectum</th>
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<td>3</td>
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<td>Ab</td>
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<td>Ab</td>
<td>(2.2)</td>
<td>Ab, Ac, (3.5)</td>
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<td>4</td>
<td>—</td>
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<td>Ab</td>
<td>Ab</td>
<td>(1.9)</td>
<td>Ac, Ac, As (4.2)</td>
<td>Ac, Ac, As (3.1)</td>
<td>Ac</td>
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<tr>
<td>5</td>
<td>—</td>
<td>—</td>
<td>Ab</td>
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<td>(1.9)</td>
<td>Ac</td>
<td>(1.9)</td>
<td>Ac</td>
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<td>Ab, Ac, Ac (4.9)</td>
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<tr>
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<td>Ab</td>
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<td>Ab, Ac, Ac (3.0)</td>
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⁴If log values are not given, arcobacters were only isolated after enrichment (<10² colony-forming units [CFU] g⁻¹). —, no isolation of arcobacters; Ab, *Arcobacter butzleri*; Ac, *Arcobacter cryaerophilus*; Aci, *Arcobacter cibarius*; As, *Arcobacter skirrowii*; At, *Arcobacter thereius*.

Five animal associated species were present in the pigs (Table 1). The species *A. cibarius*, *A. thereius*, and *A. skirrowii* were only recovered after direct plating, but both *A. cryaerophilus* and *A. butzleri* were also isolated after enrichment. In general, only one *Arcobacter* species (either *A. butzleri* or *A. cryaerophilus*) was isolated from the content of the stomach and small intestine of the pigs. The highest *Arcobacter* species diversity was present in the large intestine and, except for pigs 4 and 5, in the rectum. *Arcobacter cryaerophilus* and *A. butzleri* were isolated at least once from all gastrointestinal sections of the pigs. *Arcobacter cibarius* was recovered from the rectal content of pigs 7, 8, 10, and 12, and the colon descendens of pig 9. Pigs 2, 6, and 10 had *A. thereius* in the rectal content, and *A. skirrowii* was only present in the caecal content of pig 4. One animal (pig 10) was infected with four *Arcobacter* species simultaneously (Table 1). Only one pig (1) carried a single *Arcobacter* species. Except for the rectal content of pig 11, the species isolated from the mucus were identical to those from the corresponding content.

In the present study, 384 *Arcobacter* isolates were characterized by ERIC-PCR, and 179 genotypes were distinguished. Genotypes with the same or similar banding patterns by ERIC-PCR analysis and present in more than one gastrointestinal section were also typed by PFGE. The *Arcobacter* strain distribution within each pig is shown in Table 2. In most cases, PFGE analysis confirmed the presence of 131 *A. cryaerophilus*, 30 *A. butzleri*, 14 *A. cibarius*, three *A. thereius*, and one *A. skirrowii* strains. Additional restriction analysis with *Nru*I always confirmed the results obtained with *Kpn*I. Figure 1 presents the banding patterns obtained with ERIC-PCR (Fig. 1A) of an identical *A. butzleri* genotype consisting of isolates from the content of the stomach, duodenum, caecum, colon ascendens, colon descendens, and rectum of pig 3. However, an additional fragment was observed for the caecal isolate (pig 3, caecum E) after PFGE analysis with both restriction enzymes (Fig. 1B,C). Another indistinguishable *A. cryaerophilus* genotype consisting of isolates from the stomach, duodenum and colon ascendens was obtained from pig 8, by ERIC-PCR. After PFGE with both restriction enzymes one duodenal isolate lacked a fragment possessed by the other strains (data not shown). In the present study, a certain *Arcobacter* strain was only present in the gastrointestinal tract of one pig. In many times, these strains were also recovered from a single specific section of the gastrointestinal tract only. Despite the fact that *Arcobacter* strains were mostly detected in one gastrointestinal region, several strains were recovered from the content of various gastrointestinal sections in eight pigs (Table 2, marked with an underscore). In total, four *A. butzleri* and nine *A. cryaerophilus* strains were isolated from the gastrointestinal content of up to six and three gastrointestinal sections, respectively (Table 2). In addition, from both the content and mucus of the same gastrointestinal sections of individual pigs, three *A. butzleri*, five *A. cryaerophilus*, and one *A. cibarius* strains were recovered. The greatest strain diversity was detected in the rectal content.

**Discussion**

Pigs are important reservoirs for *Arcobacter* species (Hume *et al.*, 2001; Van Driessche *et al.*, 2003), and their excretion in porcine feces has frequently been reported (De Smet *et al.*, 2011b; Van Driessche *et al.*, 2004). However, information on their spatial distribution in the porcine gastrointestinal tract has been lacking. In the present study, *Arcobacter* was isolated from at least two gastrointestinal sections of all 12 pigs in numbers up to 10⁷ CFU g⁻¹. The absence of *Arcobacter* in certain sections can be explained either by the possibility that they really were absent or that their numbers were below the detection limit of the isolation protocol applied. Both *A. butzleri* and *A. cryaerophilus* were isolated from the content of all gastrointestinal sections, in contrast to *A. cibarius*, *A. thereius*, and *A. skirrowii*. Van Driessche *et al.* (2003) suggested that the
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<td>B-23</td>
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The same genotype numbers in the content and/or mucus (which are underlined) of different gastrointestinal sections of an individual pig, correspond to the presence of similar genotypes in these gastrointestinal sections.


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**Table 2. Distribution of Arcobacter Strains in the Different Sections of the Gastrointestinal Tract**
isolation method used can bias the study outcome because certain Arcobacter species may be better adapted to the isolation media and procedure, especially when an enrichment step is applied. However, this possibility was excluded from this study as both a direct isolation and enrichment of a previously validated Arcobacter isolation method were applied (Houf et al., 2001; Vandamme et al., 1991; Van Driessche et al., 2003).

Some A. butzleri and A. cryaerophilus strains were isolated from every section of the gastrointestinal tract. In an Arcobacter infection study with 1-day-old piglets, A. butzleri was also the longest excreted species in the feces (up to 10 days post-infection), suggesting an intestinal colonization and multiplication, in contrast to A. skirrowii and A. cryaerophilus, which displayed a shorter duration of shedding (Wesley et al., 2001; Vandamme et al., 1991; Van Driessche et al., 2003).

FIG. 1. Characterization of the Arcobacter butzleri isolates present in the content of six sections (stomach, duodenum, caecum, colon ascendens, colon descendens, and rectum) of the gastrointestinal tract of pig 3 by enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) (A) and pulsed-field gel electrophoresis (PFGE) [restriction enzymes KpnI (B) and NruI (C)]. An additional fragment (indicated by an arrow) was obtained for one of the isolates (three caecum E) by PFGE with the restriction enzymes KpnI and NruI. (A) The ERIC-PCR clusters were constructed using the Pearson product moment correlation coefficient with 1% position tolerance, and the unweighted pair group linkage analysis method (UPGMA). (B,C) The PFGE pattern clusters were constructed using the Dice coefficient and UPGMA. D, isolate obtained after direct plating; E, isolate obtained after enrichment.
**References**


De Smet S, Vandamme P, De Zutter L, On SLW, Douidah L, Hufnau J, Houf K. *Arcobacter* contamination during processing. However, due to the great heterogeneity within an animal, typing *Arcobacter* isolates seems not to contribute to the identification of initial contamination sources. Furthermore, it could not be elucidated whether *Arcobacter* or some particular strains truly colonize the intestines of pigs or are just passengers of the gastrointestinal tract. These pathways should probably be taken into account, as evidence for both have been demonstrated. However, this means that pigs are constantly being infected by arcobacters present in their environment. For the latter, this could not be confirmed in a previous study on the epidemiology of *Arcobacter* during the fattening period (De Smet et al., 2011b).

**Acknowledgments**

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**Disclosure Statement**

No competing financial interests exist.
ARCOBACTER IN THE GASTROINTESTINAL TRACT OF PIGS 1103


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