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Arcobacter Population Dynamics in Pigs on Farrow-to-Finish Farms▽†

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Healthy pigs are an important reservoir for the emerging human pathogen *Arcobacter* which can result in contamination of porcine carcasses and pork and the spread of arcobacters into the environment. Up to now, the excretion of arcobacters by pigs has been studied, but information about the transmission routes in fattening pigs is lacking. The present study aimed to elucidate the *Arcobacter* population dynamics in pigs during the fattening period on four farrow-to-finish farms. On each farm, 30 clinically healthy, 12-week-old piglets were selected. Fecal samples were collected on 10 sampling occasions until a slaughter age of 30 weeks was reached. *Arcobacter* spp. were isolated by a selective method and identified by multiplex PCR. The genetic diversity was examined by amplified fragment length polymorphism and enterobacterial repetitive intergenic consensus PCR. The *Arcobacter* presence in the fecal samples on the four farms ranged from 11.3 to 50.0%, with excretion levels of up to 10^4 CFU/g feces. The ratio in which *Arcobacter* species were isolated varied between the farms and over time. Characterization revealed a high degree of genotypic diversity among the isolates. *Arcobacter* strains persisted and spread within the finishing unit during the fattening period. The occurrence of both unique and shared genotypes in pigs in adjacent and nonadjacent pens demonstrates that transmission routes other than fecal-oral transmission occur.

In the late 1970s, the first isolation of aerotolerant campylobacters from aborted porcine fetuses was reported (10, 29). These organisms, for which the genus *Arcobacter* was created in 1991, are closely related to campylobacters but are able to grow in air and at temperatures of below 30°C (40). At present, nine species are characterized, of which five are animal related (3, 21, 27). In humans, *Arcobacter cryaerophilus* and *Arcobacter skirrowii* have been isolated from diarrheal stool samples (26, 35, 52), but predominantly *Arcobacter butzleri* has been associated with enteritis and septicemia (19). Moreover, *A. butzleri* was the fourth most common *Campylobacteraceae* species isolated in both a Belgian survey and a French survey (32, 42).

Contaminated drinking water has been identified as a major source of infection in developing countries (39). In industrialized countries, human infection is assumed to be food borne and probably occurs through the manipulation, consumption, or cross-contamination of raw and undercooked meat products. Close contact with pets and person-to-person contact are other potential risk factors for human infection (11, 20, 41). Arcobacters are commonly present in food of animal origin, with the highest prevalence reported for poultry, pork, and beef (7, 25, 34, 37, 46, 47). The origin of the contamination in poultry products is still debated, but fecal material is regarded as the main source of pork and beef carcass contamination (47).

In the past decade, *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* have been isolated from the feces of healthy pigs at slaughter age (14, 15, 23, 24, 43). However, they have also been implicated in reproduction disorders as late-term abortions and a higher rate of stillbirths and have been isolated from internal organs of aborted pig fetuses and placental, uterine, and oviductal tissues (5, 10, 36). They are present in vaginal swabs of normal producing sows and were isolated from preputial fluid of boars on farms with reproductive problems, though they have not been detected in the semen (6, 24). Nevertheless, insemination with experimentally infected semen seemed to induce a decrease in conception rates in sows.

Hitherto, intrauterine and horizontal transmission between sows and their piglets up to 3 weeks old has been studied (15), but there is no information about the transmission in pigs during the fattening period. Elucidation of the infection sources and transmission of arcobacters in fattening pigs is essential to develop intervention strategies for reducing *Arcobacter* infection at slaughter age and eventually to prevent carcass and meat contamination during slaughter. Hence, a longitudinal study on four Belgian farrow-to-finish farms was undertaken to explore the *Arcobacter* epidemiology within fattening pigs from 12 to 30 weeks old.

MATERIALS AND METHODS

Study setup. Four unrelated farrow-to-finish closed farms (farms A, B, C, and D) situated in the northern part of Belgium were randomly selected to participate in the present study. Details on farm management are shown in Table 1. Animals were accommodated with an average of 12 piglets per pen, and per farm 30 piglets of 12 weeks of age (both neutered male and female) were randomly selected. Ten piglets were randomly chosen in a first pen (pen 1), 10 in an adjacent second pen (pen 2), and another 10 in a nonadjacent third pen (pen 3) on the other side of the central passageway in the opposite corner of the pigsty

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TABLE 1. Farm data

Parameter	Farm A	Farm B	Farm C	Farm D
Breed	Hypor sow × Piétrain boar	French hybrid sow × Piétrain boar	Topics 20 sow × Piétrain boar	Topics 20 sow × Piétrain boar
No. of sows	160	180	700	700
No. of piglets (0–12 wk old)	680	470	1,890	3,000
No. of fattening pigs (12 wk old to slaughter)	900	1,200	6,500	5,000
Visible presence of rodents in pigsty	No	Yes	No	No
Possibility of presence of dogs or cats in pigsty	Yes	No	No	No
Presence of wet slatted floors in pigsty	No	Yes	No	No
Practice of tail biting	No	Yes	No	No
Application of drinking water medication	Yes	Yes	Yes	No
Ventilation system in finishing unit	Ceiling ventilation	Natural ventilation	Mechanical flap ventilation	Channel ventilation
Type of feed in finishing unit	Meal	Meal	Meal	Meal

(Fig. 1). Direct contact between animals was possible only in the adjacent pens 1 and 2, through the partially opened side wall. Fecal samples from the individually ear-tagged pigs (pigs 1 to 120) were collected rectally every 2 weeks using sterile gloves, starting at 12 weeks of age (around 20 kg) until the slaughter age of approximately 30 weeks (around 110 to 115 kg). Samples were transported under cooled conditions to the laboratory and were always processed within 4 h. Samplings took place from February until June (farms A and B), from April until August (farm C), and from May until September 2007 (farm D). Each animal and its fecal samples were represented by an unique code: farm (A to D)/sampling occasion (1 to 10)/pen (1 to 3)/pig number (1 to 120). In total, 1,100 fecal samples were collected on the four farms during the sampling period, with 282, 256, 266, and 296 samples taken on farms A, B, C, and D, respectively. The difference in the number of samples is due to occasional absence of fecal material in the rectum, death of certain animals, or selling of pigs. Doxycycline (doxycycline hyclate; 500 mg/g) was incorporated in the drinking water for 5 days, preventively between sampling points 2 and 3 on farm B and curatively (for cough) between sampling occasions 4 and 5 and sampling occasions 6 and 7 on farms A and C, respectively.

The statistical analysis of the differences in *Arcobacter* excretion within the sampling occasions on each farm and between the farms was performed with the chi-square test.

***Arcobacter* isolation, identification, and characterization.** For the selective isolation of arcobacters, 5 g feces was homogenized in 45 ml *Arcobacter* selective isolation broth (containing 24 g liter^{-1} *Arcobacter* broth [CM 965; Oxoid, Basingstoke, United Kingdom], 100 mg liter^{-1} 5-fluorouracil [F6627; Sigma, St. Louis, MO], 100 mg liter^{-1} cycloheximide [C7698; Sigma], 10 mg liter^{-1} amphotericin B [A4888; Sigma], 16 mg liter^{-1} cefoperazone [C4292; Sigma], 32 mg liter^{-1} novobiocin [N1628; Sigma], 64 mg liter^{-1} trimethoprim [T0667; Sigma], and 50 ml liter^{-1} lysed defibrinated horse blood [E&O Laboratories Ltd., Bonnybridge, Scotland]) (43) using a stomacher blender (IUL Instruments, Barcelona, Spain) for 1 min at normal speed. For the quantitative analysis, 100 μl of each homogenate was inoculated onto an *Arcobacter* selective isolation agar plate (containing 24 g liter^{-1} *Arcobacter* broth, 12 g liter^{-1} Agar Technical No. 3 [LP0013; Oxoid], and the selective supplements described above) (43) using the spiral plating technique (Eddy Jet; IUL Instruments). Both plates and 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 a jar. Following incubation, the plates were checked for typical bluish colonies using Henry transillumination and counted. In addition, 100 μl of each incubated homogenate was streaked onto an *Arcobacter* selective agar plate and incubated under the same conditions as described above. A maximum of 10 colonies were randomly picked from the plates used for counting, and 1 colony was picked from the plates after selective enrichment. The isolates were subcultured once on blood agar plates (containing 24 g liter^{-1} *Arcobacter* broth, 12 g liter^{-1} Agar Technical No. 3, and 50 ml liter^{-1} defibrinated horse blood) and stored in 500 μl defibrinated horse blood at −80°C until further identification and characterization. Template DNA was extracted by suspending the bacterial cells in 0.5 ml RS buffer (1,753 g NaCl and 744 mg EDTA adjusted with sterile water to a final volume of 200 ml [pH 8.0]). The suspensions were centrifuged for 2 min at 17,400 × *g* (Eppendorf 5417-R cen-

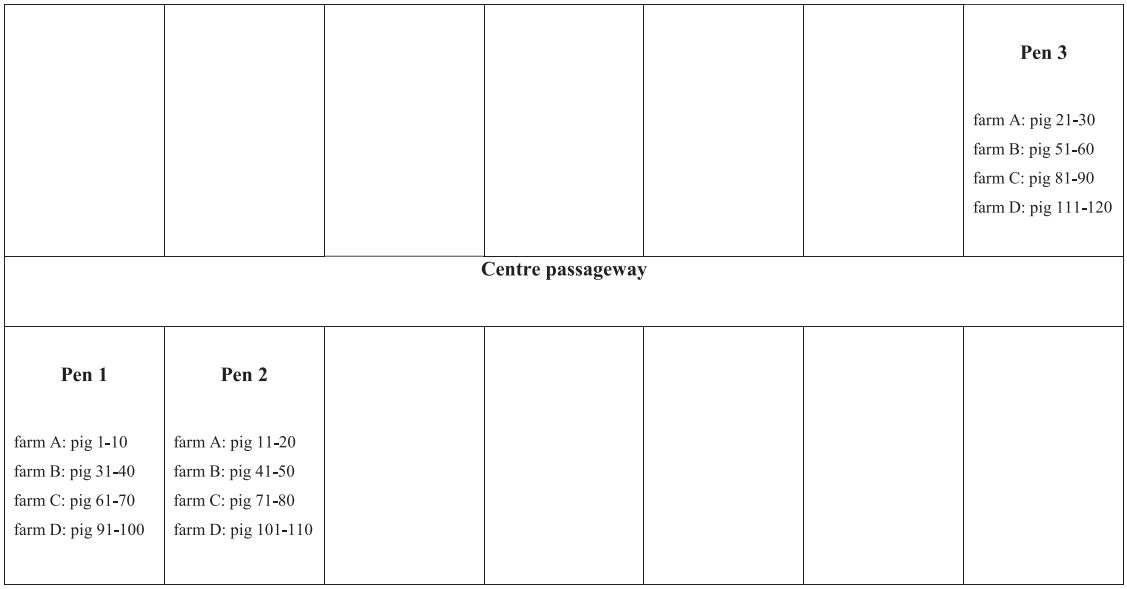

FIG. 1. Floor plan of the finishing unit on the farms.

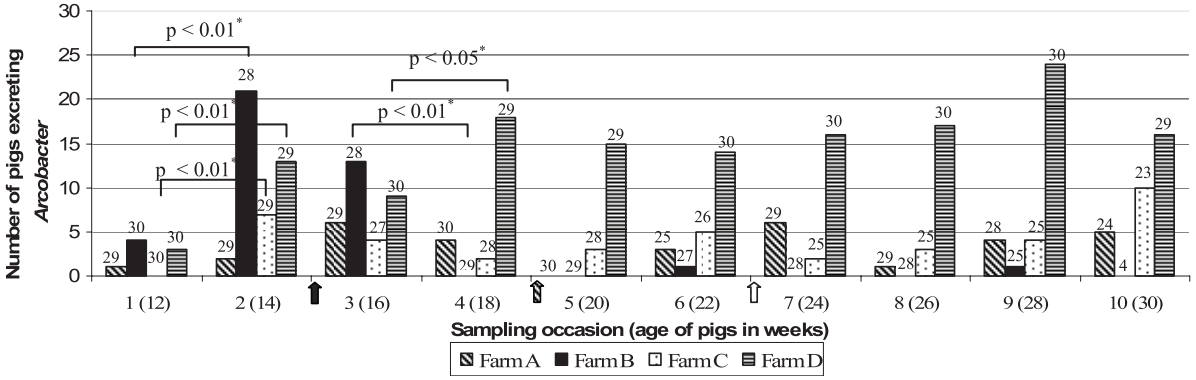

FIG. 2. Number of pigs shedding *Arcobacter* in the feces as a function of the age of the animals on the four farms. Arrows indicate the application of doxycyclin on farms A, B, and C. Numbers above bars indicate the number of fecal samples collected per sampling occasion. *, significant variation in *Arcobacter* excretion between consecutive sampling occasions.

trifuge; Eppendorf, Hamburg, Germany) to pellet the cells, and the supernatant was discarded. The pellets were then resuspended in 100 μl Tris-EDTA buffer, and genomic DNA was extracted by the guanidium thiocyanate method (31). The concentration of each DNA template was determined spectrophotometrically (Bio Photometer; Eppendorf) at 260 nm and adjusted to about 50 ng μl^{-1}. Two microliters was used in the *Arcobacter* species-specific multiplex PCR (m-PCR) assay described by Douidah et al. (9). Isolates for which no species-specific band in the multiplex PCR was generated were further examined using the *Arcobacter* genus-specific PCR described by Harmon and Wesley (13).

Subsequently, the isolates were characterized at the strain level by two typing methods. For amplified fragment length polymorphism (AFLP) analysis, the protocol described by Debruyne et al. (4) was used. DNA integrity was controlled by 1.5% agarose–Tris-borate-EDTA (TBE) gel electrophoresis for 30 min at 70 V. One microgram of chromosomal DNA was simultaneously digested with the restriction enzymes HindIII and HhaI (New England BioLabs, Ipswich, MA). Restriction site-specific HindIII and HhaI adapters were ligated to the restriction fragments, after which a selective PCR with a fluorescence-labeled (6-carboxy-fluorescein [FAM]) HindIII primer (5′-GACTGCGTACCAGCTT-3′) and a HhaI primer (with an additional 3′A nucleotide, 5′-GATGAGTCCTGATCGC A-3′) was carried out. One microliter of the final product was mixed with 8.6 μl of deionized formamide and 0.4 μl of the internal lane standard (Gene Scan-600 LIZ size standard; Applied Biosystems) and analyzed by means of capillary electrophoresis using an ABI 3130xl Genetic Analyzer (Applied Biosystems). AFLP profiles were collected with the data collection software 3.0 (Applied Biosystems). Computer-based normalization and interpolation of the DNA profiles and numerical analysis using the Pearson product moment correlation coefficient (1% position tolerance) were performed using the BioNumerics v. 4.61 software package (Applied Maths, Sint-Martens-Latem, Belgium). The correlation level was expressed as a percent similarity. Dendrograms were constructed using the unweighted-pair group method using average linkages (UPGMA).

For enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) (17), 2 μl DNA (about 50 ng μl^{-1}) was added to a 48-μl PCR mixture containing the ERIC primers 1R (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and R2 (5′-AA GTAAGTGACTGGGGTGAGCG-3′) (48) at a concentration of 25 pmol each. The PCR products were size separated by electrophoresis of 10 μl of the reaction volume in 2% agarose-TBE gels for 2 h at 100 V. For interpretation of the fingerprints, the GelCompare 4.2 software package (Applied Maths) was used. 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. For convenience, the correlation level was expressed as a percent similarity. Dendrograms were constructed using UPGMA. DNA patterns that differed by one or more DNA fragments were considered different genotypes (2, 18, 44, 46).

RESULTS

Farm level. Arcobacters were present on all four farms. In total, 92 of the 120 pigs excreted arcobacters at least once during their lifetimes (19, 23, 21, and 29 animals on farms A, B, C, and D, respectively). None of the pigs excreted arcobacters throughout the whole study period of 18 weeks. Fifty-one pigs shed arcobacters only once during the finishing period. Of the 41 pigs shedding arcobacters more than once, 24 excreted arcobacters during consecutive sampling occasions, while another 17 shed arcobacters during nonconsecutive samplings in the study period. Significant variations in the number of the excreting pigs were observed between the farms ($P < 0.01$) and on certain sampling occasions ($P < 0.05$) on farms B, C, and D (Fig. 2).

In total, 52 pigs excreted arcobacters at levels of up to 10^4 CFU/g feces and 40 pigs shed *Arcobacter* at levels lower than 10^2 CFU/g feces. The *Arcobacter* counts in subsequent samples collected per animal showed a fluctuating pattern.

During the sampling period, arcobacters were not isolated from the gastrointestinal contents of eight rats present in the finishing unit of farm B (data not shown).

At three farms (A, B, and C), doxycycline hyclate was incorporated in the drinking water for 5 days during the course of the finishing period (Fig. 2). On the sampling occasion following the use of the antimicrobial agent, a nonsignificant ($P > 0.05$) decrease in the number of pigs excreting arcobacters was observed.

Arcobacter butzleri, *A. cryaerophilus*, *A. skirrowii*, and *A. thereius* were isolated on farms A and C, while *A. butzleri*, *A. skirrowii*, and *A. thereius* were recovered on farm B. On farm D, *A. butzleri*, *A. cryaerophilus*, and *A. thereius* were present (Table 2). In addition, 16 isolates were not identified using the m-PCR assay but generated a genus-specific fragment. AFLP analysis revealed 10 profiles among these isolates which clustered distinctly from the established *Arcobacter* species (Table 2 and data not shown). A taxonomic study published elsewhere demonstrated that these 16 isolates represent a novel *Arcobacter* species for which the name *A. trophiarum* sp. nov. has been proposed (8).

Surprisingly, *A. thereius* was the species most commonly isolated on farms A and B (excreted by 10 and 18 animals, respectively) (see Fig. S1 in the supplemental material). On the other hand, the majority of the isolates from farms C and D was identified as *A. butzleri*. Moreover, *A. butzleri* was isolated from all pigs on farms C and D that excreted arcobacters at

TABLE 2. *Arcobacter* isolation load and species level identification results

Farm	% Isolation (total no. of fecal samples collected) by:		No. of isolates identified (no. of isolates obtained after direct plating/no. of isolates obtained after enrichment)				
	Direct plating	Enrichment	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*	*A. thereius*	*A. trophiarum* sp. nov.
A	4.6 (282)	11.3 (282)	10 (1/9)	3 (0/3)	1 (0/1)	50 (47/3)	1 (0/1)
B	8.6 (256)	15.6 (256)	10 (0/10)	0 (0/0)	37 (36/1)	78 (68/10)	15 (4/11)
C	4.1 (266)	15.0 (266)	44 (10/34)	2 (1/1)	1 (1/0)	1 (0/1)	0 (0/0)
D	11.1 (296)	50.0 (296)	215 (78/137)	7 (5/2)	0 (0/0)	10 (7/3)	0 (0/0)

least once during the sampling period. One pig each on farms A and C and 11 pigs on farm B excreted *A. skirrowii*. *Arcobacter cryaerophilus* was isolated from three, one, and five pigs on farms A, C, and D, respectively.

Arcobacter thereius and *A. skirrowii* were mostly isolated after direct plating and were consistently shed at levels higher than 10^2 CFU g^{-1} feces (Table 2). The majority of the *A. butzleri* and *A. trophiarum* sp. nov. isolates were recovered after enrichment and were shed at levels lower than 10^2 CFU g^{-1} feces. Except for one occasion, *A. cryaerophilus* was isolated at levels lower than 10^2 CFU g^{-1} feces.

At a similarity level of 85% used to discriminate genotypes, 110 AFLP genotypes were distinguished among all picked isolates (4). Isolates within these AFLP genotypes were often distinguishable by ERIC-PCR, and a total of 219 ERIC-PCR genotypes were obtained (Table 3). Two *A. butzleri* AFLP genotypes and one *A. cryaerophilus* AFLP genotype were found on two farms (C and D); in addition, one *A. thereius* AFLP genotype was present on farm A as well as on farm B. The majority (78/110) of the AFLP genotypes on the four farms was detected only once on a sampling occasion; the remaining 32 AFLP genotypes were isolated on multiple sampling occasions. Seventeen AFLP genotypes (farm A, 7/8; farm B, 1/8; farm C, 3/4; and farm D, 6/12) occurring more than once were excreted intermittently. Eleven AFLP genotypes (farm A, 1/8; farm B, 7/8; farm C, 1/4; and farm D, 2/12) were excreted on a number of consecutive samplings and then disappeared, and four AFLP genotypes on farm D were present predominantly during the finishing period and were shed on 9 (*A. butzleri* AFLP genotype B1 [18 pigs]), 8 (*A. butzleri* AFLP genotype B4 [15 pigs] and *A. butzleri* AFLP genotype B8 [17 pigs]), and 6 (*A. butzleri* AFLP genotype B11 [12 pigs]) sampling occasions.

Sixty-eight percent (75/110) of the AFLP genotypes occurring on the farms were excreted by the pigs at levels of between 10^2 and 10^4 CFU g^{-1} feces, while the other 35 *Arcobacter* AFLP genotypes were shed at levels below 10^2 CFU g^{-1} feces.

Pen level. Arcobacters were recovered from fecal samples in all pens. The *Arcobacter* species distribution at the pen level on the four farms is shown in Fig. S1 in the supplemental material. On farms A and B, *A. thereius* and *A. butzleri* were excreted in all three pens. *Arcobacter butzleri* and *A. thereius* were isolated from the feces of 8 and 10 pigs, respectively, on farm A. On farm B, 7 and 18 pigs shed *A. butzleri* and *A. thereius*, respectively. *Arcobacter skirrowii* was isolated from the feces of 11 pigs housed in pens 1 and 2 on farm B, but on farm A this species was found in only one pig (A/3/2/17) accommodated in pen 2. In pen 1 (A/1/1/7), pen 2 (A/4/2/17), and pen 3 (A/3/3/26) of farm A, *A. cryaerophilus* was excreted by one animal. On the other hand, *A. cryaerophilus* was not isolated on farm B. On farm A, *A. trophiarum* sp. nov. was excreted by one pig (A/1/1/24) in the first pen, and on farm B this species was isolated from 12 pigs housed in pens 1 and 2. *Arcobacter butzleri* was isolated from 21 pigs housed in either pen 1, 2, or 3 on farm C. Conversely, *A. cryaerophilus* (C/2/1/64 and C/3/1/64), *A. thereius* (C/10/3/88), and *A. skirrowii* (C/7/3/87) were excreted by only one pig in either pen 1 or pen 3 on farm C. On farm D, *A. butzleri* and *A. cryaerophilus* were present in all pens and were shed by 29 and 5 pigs, respectively. *Arcobacter thereius* was excreted by three pigs (D/2/1/91, D/4/1/91, D/2/1/100, and D/1/2/109) in the first and second pens. *Arcobacter skirrowii* was not isolated on farm D.

With the exception of farm B, no clear difference in the number of shared genotypes in pigs of adjacent pens (pens 1 and 2) in comparison with animals housed in nonadjacent pens (either pen 1 versus 3 or pen 2 versus 3) was found. On all farms, the majority (farm A, 21/25; farm B, 33/45; farm C, 10/13; and farm D, 16/27) of the AFLP genotypes were found in only one pen and were mostly excreted by only one animal (farm A, 17/21; farm B, 25/33; farm C, 9/10; and farm D,

TABLE 3. Number of *Arcobacter* genotypes as determined by AFLP and ERIC-PCR

Farm	No. of genotypes determined by:							
	AFLP				ERIC-PCR			
	A. butzleri	*A. cryaerophilus*	*A. skirrowii*	*A. thereius*	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*	*A. thereius*
A	5	3	1	16	7	3	1	36
B	3	—[a]	15	27	5	—	28	57
C	9	2	1	1	13	2	1	1
D	20	4	—	3	55	4	—	6

[a] —, not isolated.

14/16). Conversely, one (farms A and B), two (farm C), and seven (farm D) *A. butzleri* AFLP genotypes were excreted in two pens. On farms A, B, and D, three, seven, and one *A. thereius* AFLP genotype, respectively, were recovered from pigs in two pens. In addition, one *A. cryaerophilus* AFLP genotype (C1, farm D) and two *A. skirrowii* AFLP genotypes (S9 and S12, farm B) were shed by pigs housed in pens 1 and 2. Two genotypes present on farms B (*A. butzleri* AFLP genotype B2 and *A. thereius* AFLP genotype T2) and D (*A. butzleri* AFLP genotypes B1 and B4) and one *A. butzleri* AFLP genotype (B7) on farm C were excreted by pigs in all three pens examined.

Animal level. The majority (57/92) of the animals excreted only one *Arcobacter* species at a time during the whole fattening period. The other pigs mostly shed two or three species in the course of the fattening period. Pig 36 excreted four *Arcobacter* species (*A. thereius*, *A. skirrowii*, *A. butzleri*, and *A. trophiarum* sp. nov.), and at 14 weeks of age, the species *A. thereius*, *A. skirrowii*, and *A. butzleri* were shed.

During the fattening period, 60 of the 92 pigs excreted more than one AFLP genotype per species. A large majority of these genotypes (78/110) were detected on one sampling occasion and were excreted mostly by one animal (65/110). On one sampling occasion, pigs maximally excreted five *A. butzleri* (D/4/2/107), eight *A. thereius* (B/2/1/36), and five *A. skirrowii* (B/2/2/45) genotypes or one *A. cryaerophilus* (for example, A/7/1/7, C/2/1/64, and D/8/2/110) genotype. However, the other 32 genotypes were excreted on several sampling occasions. For example, *A. butzleri* genotype B8 (farm D) was excreted by pig 99 on four successive samplings (D/6/1/99, D/7/1/99, D/8/1/99, and D/9/1/99). Both on farm A (*A. thereius* genotypes T9 and T11) and on farm B (*A. thereius* genotypes T2 and T19), two *A. thereius* genotypes were each recovered from the feces of one pig during two successive samplings. The *A. cryaerophilus* and *A. skirrowii* genotypes were shed only on one successive sampling occasion by one pig.

DISCUSSION

Arcobacters are commonly present in food of animal origin, with the highest prevalence reported for poultry, followed by pork (47). However, in contrast to the case for poultry, arcobacters are associated with pigs throughout the production chain, from piglet to ground meat. Information on the *Arcobacter* population dynamics during the fattening period, however, is lacking (15, 43, 44).

In the present study, 92 of the 120 pigs excreted arcobacters at least once during the finishing period without any clinical symptoms or notable influence on the rearing parameters. Arcobacters also do not seem to be part of the essential commensal intestinal flora, as the *Arcobacter* excretion was highly dynamic in time, numbers, and strains. This intermittent excretion and strain variability can be explained by temporal clearance followed by reinfection, by a resting phase of the bacteria through an accumulation at the bottom of the crypts in the intestinal mucosa, or by a tendency to accumulate in the rectal mucus, resulting in a heterogeneous distribution in the gut content (22, 28). Moreover, in contrast with previous findings (23, 44), the number of excreting animals and the number of arcobacters in the feces did not increase with the age of the animals.

Notwithstanding the variable species and genotype composition in and between animals during the fattening period in the present study, several *Arcobacter* transmission routes were identified. Although strains were mostly recovered from one animal only, transmission of the same strain between animals housed in the same pen and pigs in both adjacent and nonadjacent pens was demonstrated. On farm D, for example, *Arcobacter butzleri* genotype B4 was first excreted by pigs 109 (D/2/2/109) and 110 (D/2/2/110) at 14 weeks of age, after which it spread in pen 2 (pigs 102, 105, 106, 107, and 108) during the following samplings. From the fifth sampling occasion (20 weeks of age), this genotype was further recovered from seven pigs (111, 112, 115, 116, 117, 119, and 120) in pen 3 as well. In pen 1, only animal 95 (D/9/1/95) shed *A. butzleri* genotype B4 at 28 weeks of age (see Fig. S2 in the supplemental material). The presence of the same strain within the same pen and in adjacent pens can be explained by fecal-oral transmission during close contact through the partially opened side walls. Oral uptake of arcobacters followed by fecal excretion has previously been observed during an experimental *in vivo* study in 1-day-old piglets (51) and in piglets up to 3 weeks of age (15). The occurrence of unique *Arcobacter* strains in nonadjacent and adjacent pens demonstrates that other infection routes, such as contaminated water, rodents, the pig farmer, and iatrogenic transmission, are probably important (44, 45). In contrast to the case for *Campylobacter* (1), *Arcobacter* transmission through rodents could not be confirmed in the present study, as the gastrointestinal tracts of the rats examined did not harbor *Arcobacter* (data not shown).

As reported for *Campylobacter coli* in pigs (38, 49, 50), some *Arcobacter* strains spread over the pig house, resulting in dominant strains in several animals. For example strains B1, B4, B8, and B11 were predominantly excreted by several pigs throughout the finishing period on farm D. The dominance of such strains may suggest a greater capacity to survive the environmental stresses and to colonize the host gut, as previously described for *Campylobacter jejuni* (12, 33). Differences in colonization types can be due to genetic differences or differences in expression of colonization and invasion related genes (12).

The large heterogeneity of *Arcobacter* populations, in both species and strain composition, has been discussed almost since the first description of the genus in 1992. Like for *Campylobacter* (33, 49), multiple parent genotypes from different infection sources and genomic rearrangements and DNA uptake from the environment have been suggested as the driving force for this diversity, but none of these hypotheses have been firmly confirmed. Apart from the biological variation, the isolation and typing method also can bias the outcome of diversity studies. The strains recovered may reflect a superior adaptation to the isolation procedure and medium components rather than their dominant occurrence, especially when an enrichment step is applied. In the present study, a previously validated *Arcobacter* isolation method with both direct isolation and isolation after enrichment was applied (43). In contrast to other *Arcobacter* isolation methods, the composition of the selective supplement in the medium allows growth of all currently known mammal-associated species with a maximum suppression of the accompanying flora (16). The lower rates of

isolation of *A. cryaerophilus* and *A. skirrowii* can be explained either by a lower occurrence in the intestinal tract or by the more fastidious growth requirements of those species (16, 43). As in previous studies, direct plating revealed a larger diversity of *Arcobacter* strains than in enrichment, where mostly one genotype was recovered (44, 45). In addition to the isolation method, the typing method also biases the results. Although no gold standard typing method has presently been established for the genus *Arcobacter*, the application of different typing methods confirms that this heterogeneity truly exists (23, 30, 44).

In the present study, a large number of *A. thereius* strains were isolated for the first time from pig feces. On farms A and B, *A. thereius* was the species most commonly excreted during the fattening period. ERIC-PCR, rather than AFLP, was most suited for *A. thereius* characterization. As reported by Debruyne et al. (4), the number of peaks observed in the AFLP profiles was significantly lower than for other *Arcobacter* species.

In conclusion, in the present study, arcobacters were isolated from the majority of animals on all farms. Although differences in farm management were observed, *Arcobacter* occurrence did not seem to be related to hygiene measurements or farm management. Moreover, within each farm, the *Arcobacter* excretion in animals is not static but is highly dynamic. The overwhelming number of strains present on each farm seriously complicates the study of *Arcobacter* epidemiology and hampers the identification of possible infection and transmission routes.

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