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Arcobacter, an ignored foodborne pathogen – a review*

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Introduction

In 1991, a taxonomic revision of all known *Campylobacter*-like organisms showed that these organisms belonged to the epsilon subdivision of the *Proteobacteria,* referred to as rRNA superfamily VI (1). DNA:rRNA hybridization studies showed the existence of three major RNA homology groups, called rRNA clusters I, II and III. Based on these data, a differentiation into four genera was proposed: the genus *Campylobacter* (rRNA cluster I), the genus *Arcobacter* (rRNA cluster II), the genus *Wolinella* (rRNA cluster III) and the genus *Helicobacter* (rRNA cluster III). Because of the close genotypic affiliation between the genera *Campylobacter* and *Arcobacter,* a new family, the *Campylobacteraceae,* was created to encompass both genera. Members of the family *Campylobacteraceae* are Gram-negative, non-spore forming rods. Cells are usually slender, 0.2 to 0.9 µm wide and 0.5 to 3 µm long. Cells in old cultures may form spherical, coccoid bodies or spiral filaments up to 20 µm long. Cells are motile with a characteristic corkscrew-like motion by means of a polar unsheathed flagellum. In general, arcobacters can be differentiated from campylobacters by their lower growth temperatures and aerotolerance (2). Within the genus *Arcobacter,* six species are presently recognized. *Arcobacter nitrofigilis, Arcobacter halophilus* and a number of yet not established species as candidatus *A. sulfidicus* form the "environmental" division (3, 4). For these species, no association with animal or human infection has yet been reported. *A. butzleri* has been associated with enteritis, abdominal cramps, bacteraemia, and appendicitis in humans and with enteritis and abortion in animals. This species has been isolated from animal tissues and faeces, from various food products of animal origin and from surface and drinking water reservoirs. The second species, *Arcobacter cryaerophilus* is regarded as a genotypically heterogeneous species. Two subgroups referred to as subgroup 1 or 1A, and subgroup 2 or 1B have been described. *A. cryaerophilus* has also been isolated from cases of human bacteraemia and diarrhoea,

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from animal faeces and food. *Arcobacter skirrowii* was designated as a third species after a comparison of aerotolerant campylobacters recovered from the faeces of lambs with diarrhoea. Additional isolates were obtained from fluid samples of preputial sheet-washings of bulls and from the internal organs of porcine, ovine and bovine aborted foetuses. Recently, it has been isolated from a human patient with enteritis (5). A fourth species, *Arcobacter cibarius* has recently been isolated and characterized as a new species from broiler carcasses (6).

Isolation of *Arcobacter* species

Arcobacters were first isolated from bovine and porcine foetuses using the semi-solid *Leptospira* isolation medium Ellinghausen, McCullough, Johnson, and Harris-polysorbate 80 supplemented with 5-fluorouracil and rabbit serum (7). Since then, several selective media and isolation protocols have been applied for the isolation of arcobacters from different matrices. Most of them are modified *Campylobacter* and even *Yersinia* protocols (8, 9), but study of the susceptibility to the selective agents included, have demonstrated there lack in the isolation of all animal related *Arcobacter* species (10). Recently, specific *Arcobacter* isolation media have been developed for the quantitative and qualitative isolation of all animal-related *Arcobacter* species from food (11) and faeces (12) with a maximal suppression of the accompanying flora.

Identification of arcobacters

Differentiating *Arcobacter* species by phenotypic tests gives erroneous results because of their metabolic inertness, resulting in a shortage of clear-cut differentiation tests, a phenomenon which has also been observed in the closely related campylobacters (2). Therefore, several DNA-based assays were developed for the identification of arcobacters at genus and species level. Nowadays, human-related arcobacters can be routinely identified within 4 hours in a single step multiplex-PCR assay (13).

Characterisation of *Arcobacter* isolates

Methods as bio- and serotyping, and SDS-PAGE of whole cell-proteins have limited discriminatory capacity, are laborious and, as typical for phenotypic tests, are variable in outcome. Therefore, characterization of arcobacters nowadays is performed by DNA-based methods as random amplified polymorphic DNA (RAPD) assay (14), pulsed field gel electrophoresis (PFGE) (15) and polymorphic DNA analysis based on genomic repetitive elements (14). Characterization of isolates in epidemiological studies, shows, as for campylobacters, a large heterogeneity at strain level (14, 15).

Arcobacter species in livestock animals

Arcobacters in bovines

The presence of *Arcobacter* species in cattle is associated with two pathologies: reproduction abnormalities and mastitis. Ellis and co-workers were the first to describe the association of arcobacters with the occurrence of abortion in cattle (7). In spite of this association, arcobacters have also been isolated from preputial sheath washing samples of bulls without any association of breeding problems in the herds (16). Moreover, arcobacters have been isolated from faecal samples of beef and dairy cattle with no signs of enteritis, abortion or reproduction abnormalities (17, 18). Therefore, the role of arcobacters in the aetiology of abortion remains unclear. Only two studies have reported the association between arcobacters and mastitis so far. In 1982, *Logan et al.* isolated arcobacters from raw milk of an outbreak of mastitis in a dairy herd. The isolates induced clinical mastitis in cows experimentally infected by intramammary inoculation (19). Markedly swollen and painful quarters within four hours of inoculation, elevated pulse rate and temperature and a light decline in milk production followed. Sixteen hours after infection, the infected animals showed full recovery and a return to normal levels of milk yield and cell counts.

In a recent study, from 11 % (n=276) of clinically healthy Belgian cows on three unrelated farms, *Arcobacter* have been isolated from the faeces (12, 17). Between the three farms, the *Arcobacter* prevalence ranged from 7.5 % to 15 %. The study revealed an influence of the animals' age: calves had the highest, young cattle the middle and the dairy cows the lowest *Arcobacter* colonization rate. Co-colonization was not uncommon, as more than one *Arcobacter* species was found in 26 % of the positive samples. *Arcobacter cryaerophilus* was the dominant species, followed by *A. skirrowii.* A large number of genotypes per species were determined at all farms and no genotypes were simultaneously present on different farms. In the individual animals, a large genotypic variation among the isolates was detected: the number of genotypes per species present ranged from one to nine. No indication of vertical transmission was demonstrated. *Arcobacter butzleri* genotypes present in water or on the boot were also detected in faecal samples from cows, indicating that water and farm material can be a vector for spreading arcobacters on the farm.

In conclusion, the prevalence and number of arcobacters in clinically healthy cows is rather low though the excretion of *Arcobacter* in faeces may be a potential source for carcass contamination. The initial contamination source however remains unidentified.

Arcobacters in pigs

After the first reports in the late seventies of the isolation of arcobacters from aborted pig foetuses, no additional information about these findings was reported for more than a decade (21). Renewed scientific attention came by the research of Erickson in 1992 (22). Late term abortions, repeat breeding and a higher than usual

rate of stillbirths in pigs from which *Arcobacter* species were recovered from foetal kidneys and livers were reported. Antibiotic therapy and the use of an autogenous vaccine provided limited improvement. These findings were recently confirmed in a Danish study by *On et al.* (23). As a result of the increasing number of reports, the presence of *Arcobacter* in sows and boars has been studied more extensively. Various studies have shown the presence of arcobacters in uterine and oviductal tissues and placenta samples from sows with reproductive problems, including abortions, early returns to oestrus and vulval discharges (24, 25). In addition, sows with reproductive disorders on farms with a history of *Arcobacter* associated abortion showed high antibody serum titres in the MAT test (24). In boars, arcobacters have been recovered from randomly sampled preputial swabs but not from semen samples, although insemination with experimental infected semen lowered conception rates in sows (24). Two studies have been conducted on the pathogenicity of *Arcobacter* in piglets. In the study by *Jahn et al.* (26), neonatal piglets were intraperitoneal inoculated with 10^6–10^{11} cfu arcobacters. No clinical symptoms were observed and no arcobacters were isolated from tissues *post mortem.* In a second study, the pathogenicity of *A. butzleri, A. cryaerophilus* and *A. skirrowii* was tested in caesarean-derived colostrum-deprived piglets on the basis of the duration of faecal shedding and colonization of tissues (27). The piglets experimentally infected with *A. butzleri* shed arcobacters in their faeces for up to 10 days and the microorganism was cultured from the ilea, livers, kidneys and brains. In contrast, *A. cryaerophilus* and *A. skirrowii* infected piglets showed to have a short duration of faecal shedding with no recovery of the microorganisms from diverse tissue samples on necropsy. Those latter findings suggest failure of *A. cryaerophilus* and *A. skirrowii* to penetrate the intestinal barrier.

In a study performed by *Van Driessche et al.* (20), the prevalence of *Arcobacter* in porcine faecal samples on four unrelated farms ranged from 16 % to 42 % in porkers and from 59 % to 85 % in sows. The bacterial load ranged from less than 10^2 to 10^4 cfu/g faeces. *A. butzleri* was the most frequently occurring species, but co-colonization was not uncommon as two and three *Arcobacter* species were found in 12.4 % and 3.3 % of the positive samples, respectively. Characterization of 35 *A. skirrowii,* 121 *A. cryaerophilus* and 322 *A. butzleri* isolates, distinguished 30, 70 and 123 genotypes, respectively. There were no genotypes detected on more than one farm. A large heterogeneity among the *Arcobacter* isolates was also found in an individual animal: the number of genotypes in an animal for *A. skirrowii* ranged from one to six, for *A. cryaerophilus* from one to ten and for *A. butzleri* from one to seven. *Arcobacters* were also isolated from water and boot samples, which implies a role as potential vector in the spreading of arcobacters on the farm.

In conclusion, arcobacters can be present in pigs at various prevalence and colonization levels, with a broad range in diversity of species and strains, without any clinical symptoms. The presence of *Arcobacter* in the faeces is however a potential risk for carcass contamination during slaughter.

Arcobacters in poultry

Research on the occurrence of arcobacters in chickens has been concentrated to the presence of arcobacters in the intestinal track. Although *A. butzleri, A. cryaerophilus* and *A. skirrowii* all have been isolated from poultry carcasses, it appears however that they may not be able to colonize the poultry intestinal tract (28, 29, 30, 31, 32). In a study to determine the prevalence of *Campylobacteraceae* in Belgian broilers after slaughter, broiler neck skin samples were collected at two stages of slaughter in 8 poultry slaughterhouses (30, 31). In total, 96.2 % and 95.0 % (n=480) of the samples were found positive for the presence of arcobacters, and 55.0 % and 46.2 % (n=400) for campylobacters, after evisceration and chilling respectively. Five poultry flocks were *Campylobacter*-free, but all flocks were contaminated with arcobacters, regardless of the slaughtering procedure applied or the slaughterhouse examined. In each slaughter facility, the processing line was already contaminated with arcobacters before onset of slaughter. The number of arcobacters ranged from 10^1 to 10^3 cfu/g skin. No arcobacters were recovered from the content of the 30 collected intestinal tracks.

In conclusion, poultry products are commonly contaminated with *Arcobacter butzleri* and *Arcobacter cryaerophilus* on the surfaces, though they have not convincingly been recovered from living chickens so far. Although the exact contribution of contaminated poultry products to human infection remains to be determined, the handling of raw poultry, cross-contamination and the consumption of undercooked poultry products are probable routes of transmission.

Arcobacters in water

Arcobacter nitrofigilis, Arcobacter halophilus and a number of yet not established species as candidatus *A. sulfidicus* form the "environmental" division, which is quit exceptional for *Proteobacteria* (3, 4, 33). These species have been isolated from diverse environmental sources as salt-water lakes, costal seawater, oil wells, sediments in the Black Sea and from different kind of sludge (33, 34, 35, 36, 37). Direct transmission and infection of humans and animals through the consumption of water has predominantly been reported in developing countries with insufficient water supplies. Both *A. butzleri* and *A. cryaerophilus* have frequently been isolated from drinking reservoirs in India and Thailand (38, 39). *Arcobacter butzleri* accounted for 16 % of the *Campylobacter*-like isolates obtained from Thai children with diarrhoea (38). Nevertheless, arcobacters have also been isolated from river water and water drinking reservoirs in Europe, Canada and the United States (40, 41, 42). Waterborne disease outbreaks of *Arcobacter* infection have rarely been reported so far. One outbreak of gastroenteritis occurred in July 1996 at a Girl Scout camp near Couer d'Alene, Idaho, USA. A breakdown in the camp's automated chlorination system occurred at the same time as the outbreak. It was estimated that 81 % (n=117) of the individuals at the camp became ill. Nausea and vomiting, followed by abdominal cramps and diarrhoea, were the predominant symptoms. At

that time, the local health department was unable to confirm a specific aetiological agent responsible for the outbreak, but later was the most likely causing organism identified as *A. butzleri* (42). In literature, it is often stated that *Campylobacteraceae* are vulnerable organisms, but survival studies conducted with arcobacters isolated from the same well water as the outbreak illustrated that *A. butzleri* remained viable for up to 16 days in pure well water stored at 5 °C, a temperature typical of ground water. Experiments in the study showed that disinfection practices normally used in drinking water treatment would have been adequate for controlling these organisms. However, it is important to note that, as in the case of this outbreak, continuous chlorination is essential when disinfection represents the only barrier to the spread of infectious agents via a contaminated water source (42).

Water can also function as vector, and food of animal origin can be contaminated during the slaughter process. Especially in poultry slaughter, water may even function as initial contamination source for the poultry products, as arcobacters seem not to belong to the chicken flora. *Arcobacter butzleri* en *A. cryaerophilus* were detected in the process water (scalding, picking machine and inside-outside bird washer) in several poultry slaughter plants in Belgium and the U.K. (29, 30, 31). Characterization of the isolates did not contribute to the clarification of the transmission routes as different genotypes were found on the carcasses, slaughter equipment and the process water. It remains unclear if the initial contamination source of poultry products are the chickens themselves, but with very low prevalence in the living birds, or if the (ground) water used in the plants, the reservoir tanks and even the water pipelines are causing the contamination. The risk of water functioning as source and vector for arcobacters is depending on the survival capacity in the matrix. Studies in the laboratory have shown the survival of *A. butzleri* up to 200 days in drinking water, and even longer when organic material was added to the water. In the same studies, arcobacters survived the low and even the hard scalding temperatures applied in poultry slaughterhouses, and remained viable on the carcass surface until consumption *(Van Driessche et al.,* submitted for publication). The waste water of slaughter plants are not adequate decontaminated and the application of sludge in agriculture may contribute to the spreading of the organisms in the environment, resulting in a transmission over long distances, as illustrated in the study of *Stampi et al.* (43).

Arcobacters in food of animal origin

Besides contaminated water, food of animal origin is another possible route of transmission of arcobacters to humans. Arcobacters, like thermotolerant campylobacters, have been reported more frequently from poultry than from red meats. Therefore, poultry may be a significant reservoir for infection with *Arcobacter.* It is well known that *C. jejuni* and *C. coli* frequently colonize chickens, and that poultry products are considered to be the source of most human *C. jejuni* infections. Recent studies have indicated that also arcobacters are common on broiler chicken car-

casses (28, 29, 30). Arcobacters have also been isolated from skin samples of commercially reared ducks (28) and turkeys (15, 46). Eggs do not seem to be infected (44). Apart from chickens, arcobacters have been isolated from turkey and ducks. A survey of mechanically separated turkey samples suggested that this meat may be highly contaminated by *Arcobacter* species (15, 46). Variation in the prevalence between plants was noticed, as one plant yielded 96 % of samples contaminated with arcobacters whereas arcobacters were isolated from 44 % of samples from another. Examination of duck carcasses at the abattoir revealed the presence of *A. butzleri* together with *A. cryaerophilus* and *A. skirrowii.* Ground pork, although at lower incidence, is also contaminated by *Arcobacter* species, in particular with *A. butzleri,* with varying prevalence at the processing plant level (45). During a survey of a pork-processing plant in the U.S.A., 89 % of the ground pork samples collected were contaminated with arcobacters. A survey conducted 9 months later involving the same plant and four others, only 5 % of the samples of the four plants were found to be positive for *Arcobacter,* but again, 90 % of the samples were positive from the first plant examined. It was not clear whether the sanitary practices during slaughter or the rearing of pigs on the source farms contributed to the prevalence of *Arcobacter.*

Arcobacters in humans

Arcobacter infections in humans are associated with enteritis and occasionally septicaemia. *A. butzleri* is the most commonly reported pathogen of the genus *Arcobacter.* Few reports have mentioned the isolation of *A. cryaerophilus* associated with human infection, and one case of *A. skirrowii* infection has been described so far. The most common clinical features are watery diarrhoea associated with nausea, vomiting and abdominal pain. *A. butzleri* has been isolated from several patients with severe diarrhoea (47, 48, 49, 50, 51, 52).

Most cases reported are single cases without a clear source of infection. The age of the infected patients ranged from less than 1 to 72 years. In a number of these incidents, patients suffered from underlying (chronic) diseases, which may have contributed to the progress of the infection, although *A. butzleri* infections have been reported in otherwise healthy patients. Occasionally, *A. butzleri* is associated with septicaemia. Only two outbreaks of *A. butzleri* have been documented so far. An outbreak of recurrent abdominal cramps occurred in a nursery and primary school in Italy (48). Ten of the 64 children suffered from recurrent abdominal cramps but none of them had diarrhoea. During that period, all clinical samples were collected, but only in 1992, the preserved isolates were identified as *A. butzleri.* The findings that all isolates belonged to serogroup 1 and shared identical phenotypic characteristics, protein and genotypic profiles, suggest an epidemiological relationship. The timing of the cases suggests person-to-person transmission. A second outbreak of associated gastro-enteritis happened during a Girl Scout camp in

the USA and was correlated with the breakdown of the automated water chlorination system in the camp.

In summary, the importance of *Arcobacter* species as cause of human illness seems to be small. This is may be because optimal isolation techniques have not yet established and routine primary screening procedures used for *Campylobacter* species may not allow recovery of *Arcobacter* species (50, 53). Moreover, the symptoms of *Arcobacter* infections are similar to those of campylobacteriosis and may be transient in nature, making infection numbers difficult to assess (52). Overall, arcobacters appear to be resistant to antimicrobial agents typically used in the treatment of diarrhoeal illness caused by *Campylobacter* species, such as erythromycin and other macrolide antibiotics, tetracyclines and chloramphenicol. In addition, less susceptibility to cephalosporines has also been detected (53). Optimized isolation methods are required to learn more about the pathogenesis and epidemiology of arcobacters in enteric disease in both developing and industrialized countries.

Summary

Arcobacters are Gram-negative, slender curved bacteria closely related to campylobacters. At present, six species have been characterized. *Arcobacter nitrofigilis* and *Arcobacter halophilus* are the "environmental" species whereas *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii* and *Arcobacter cibarius* are human and animal related. Though arcobacters have been associated with reproductive problems, mastitis and gastric ulcers in livestock, they have been more frequently isolated from clinically healthy animals. In humans, *Arcobacter* has been associated with abdominal illness and septicaemia. The routes of infection are still unclear, but include person-to-person contact and consumption of contaminated water and food. At present, there is no unequivocal evidence that arcobacters are hazards for human health but they have been classified by the International Committee on Microbial Specification for Foods as emerging food pathogens.

Zusammenfassung

Arcobacter spp. sind Gram-negative gekrümmte Stäbchen, die ursprünglich zur Gruppe der *Campylobacter* spp. gezählt wurden. Nach intensiven Untersuchungen wurden sie dann aber einem eigenen Genus mit heute sechs Spezies zugeordnet: *Arcobacter nitrofigilis* und *Arcobacter halophilus* werden zu den «Umweltspezies» gezählt, während *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii* und *Arcobacter cibarius* human und Tier assoziierte Spezies darstellen. *Arcobacter* spp. können beim Menschen Diarrhoe auslösen und sind in einzelnen Fällen auch assoziiert zu Septikämien beschrieben. Über die Entstehung, die Entwicklung und die Übertragungswege der Krankheiten ist bislang sehr wenig bekannt, jedoch wird eine alimentäre Übertragung mit kontaminiertem Wasser und Lebensmitteln diskutiert. *Arcobacter* spp. wurden von der ICMSF zur Gruppe der «emerging food pathogens» zugeteilt.

Résumé

Les arcobacters sont des bactéries minces et spiralées, de type Gram-négatif et proches parentes des campylobacters. A ce jour, six espèces ont été caractérisées; les espèces environnementales *Arcobacter nitrofigilis* et *Arcobacter halophilus;* ainsi que les espèces humaines et animales *Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii* et *Arcobacter cibarius.* Bien que les arcobacters ont été associées à des problèmes liés à la reproduction, de mastites et d'ulcères gastriques chez le bétail, ils ont plus fréquemment été isolés à partir d'animaux cliniquement sains. Chez l'homme, les arcobacters ont été associés à des troubles gastriques et à des septicémies. Le cycle d'infection n'est pas encore élucidé mais on sait qu'il inclue les contacts de personnes à personnes et la consommation d'aliments ainsi que d'eau contaminés. Actuellement, il n'y a pas d'évidence certaine prouvant que les arcobacters sont un danger pour la santé de l'homme. Toutefois l'International Committee on Microbial Specification for Foods a classifié les arcobacters comme germes pathogènes alimentaires émergents.

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