Research Note

Incidence of *Arcobacter* spp. in Poultry: Quantitative and Qualitative Analysis and PCR Differentiation

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MS 08-172: Received 8 April 2008/Accepted 14 June 2008

ABSTRACT

Arcobacter is part of the family *Campylobacteraceae*. As with the genus *Campylobacter*, *Arcobacter* is found responsible for human gastrointestinal infection, and it is assumed to originate from poultry meat sources. Samples from poultry slaughtering originating from a broiler slaughterhouse and a turkey slaughterhouse were analyzed for *Arcobacter*. Five broiler flocks and five turkey flocks were analyzed in the course of slaughtering and processing for the prevalence of *Arcobacter*. The prevalence in broilers was 43.0%, while turkey samples were contaminated with 18.2% of positive samples. The numbers of *Arcobacter* present on turkey skin samples ranged between 1.7 and 2.4 log CFU/cm². The prevalence changes during processing showed an increase after chilling in broilers, whereas there was a constant decrease in turkey processing. Species identification showed that all three *Arcobacter* spp. of relevance in human infection could be isolated, with *A. butzleri* being found at higher prevalence, which was followed by *A. skirrowii* and *A. cryaerophilus*.

The genus *Arcobacter* (isolated from an aborted cattle fetus) was described by Ellis et al. (7) as a gram-negative motile bacterium, which can manifest in different forms. *Arcobacter* is part of the *Epsilonproteobacteria* and belongs to the family *Campylobacteraceae* (29, 31). The genus *Arcobacter* includes four better known and three newly described species: *A. butzleri*, *A. skirrowii*, *A. cryaerophilus*, *A. nitrofigilis*, (31) *A. cibarius* (16), *A. halophilus* (5), and *A. sulfidicus* (37).

The most important distinctive feature compared with *Campylobacter* is the aerotolerance of *Arcobacter* and therefore the ability to grow aerobically (20, 30). Another feature is the growth of *Arcobacter* at temperatures as low as 15°C (11, 20, 24, 30); its optimum temperature for growth lies between 25 and 35°C. It was shown that *Arcobacter* survived low temperatures of 4°C, without considerable losses in viable counts, but storage at −20°C resulted in a reduction of two log cycles. Temperatures above 55°C led to the inactivation of *Arcobacter* within a few seconds (11).

Reports in literature show that *Arcobacter* can lead to human illness similar to *Campylobacter*. In particular, *A. butzleri* has been responsible for disease (22, 32). In addition, *A. cryaerophilus* (8, 28) and *A. skirrowii* (38) were isolated from patients with diarrhea. However, their significance for human infection is still not fully known.

Symptoms of *Arcobacter* infection from asymptomatic cases to septicemia were reported; chiefly, watery diarrhea lasting for 3 to 15 days (20, 21, 28, 32) is typical. Persistent diarrhea has been noted, as was recurrence after 2 weeks up to 2 months (38). In common cases, diarrhea is accompanied by abdominal pain and nausea. In other cases fever (sometimes marked by paroxysms of chills and sweating) and emesis were also documented (32).

In food of animal origin, *Arcobacter* was found in sources from cattle, sheep, pig, and chicken meat (3, 12, 25, 35, 36). High prevalence was shown particularly in the meat of pig and poultry origin, where poultry was even more contaminated (2, 3, 12, 19). Broiler carcasses are often contaminated with *Arcobacter* spp. In contrast to *Campylobacter*, *Arcobacter* is not usually found to be colonizing the gastrointestinal tract, but it can be isolated from slaughter equipment and from broilers prior to evisceration.

The aim of this study was to estimate the prevalence of *Arcobacter* in broiler processing, and prevalence and numbers present in turkey processing in Germany and to determine species distribution by PCR.

MATERIALS AND METHODS

In total, 305 samples of poultry were analyzed for the presence of *Arcobacter* spp. One hundred thirty-five originated from a broiler slaughterhouse, and 170 samples came from a turkey slaughterhouse. The samples were collected in northern German plants. All samples were collected over a period of 5 months (March to July), with one flock of broilers or turkeys sampled each month.

Whole broiler carcasses were collected directly from the shackles of the slaughter line before and after evisceration, and after chilling. Additionally, cut broiler parts were collected during portioning at the same plant (breasts with bone, meat and skin, and pairs of fillets). For turkey, skin samples from the neck and...
breast were collected, corresponding to the sampling locations mentioned for broilers, and an additional sampling step at prechilling was added after process elongation at the slaughterhouse for flocks designated numbers 4 and 5. The skin was cut directly from the carcasses hanging at the shackle line. During cutting at the same facility, samples of thighs, wings, medallions, and breast cuts were collected.

All samples from broiler or turkey flocks were collected at random in the course of slaughtering and processing every 10 to 25 min, depending on flock size and were put separately into sterile bags and stored refrigerated at 0 to 2°C until examination.

**Sample preparation.** Carcasses and cut broiler parts were rinsed in 500 ml of 0.9% NaCl-0.1% peptone water (Merck, Darmstadt, Germany) for 90 s, and shaken vigorously by hand. For turkey samples, 20 cm² of representative sample surface (skin and/or muscle) cut from the turkey skin or turkey portions was rinsed in 100 ml of 0.9% NaCl-0.1% peptone water for 120 s in a stomacher blender (Seward, Worthing, UK). For turkey samples, quantification was carried out by preparing decimal dilutions of the rinse and spreading 0.1-ml aliquots in duplicate onto modified charcoal–cefoperazone–deoxycholate agar plates (Oxoid, Wesel, Germany) incubated for 48 ± 2 h at 25°C.

**Qualitative analysis.** *Arcobacter* spp. were isolated as follows. One-milliliter aliquots of the carcass and skin rinse or 10 g of the cut poultry parts were added to cefoperazone–ampicillin–teicoplanin–teicoplanin broth (*Arcobacter* broth CM0965 and CAT supplement SR0174, Oxoid) at a ratio of 1:10. After aerobic incubation for 48 ± 2 h at 25°C, an aliquot was streaked onto modified charcoal–cefoperazone–deoxycholate agar and incubated at 35°C. Colonies of typical morphology were confirmed by morphological characteristics (motility, Gram stain, catalase, and oxidase tests) and identified by PCR.

**PCR.** DNA was isolated with the DNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. PCR was performed according to the method of Houf et al. (17). In short, the DNA amplification was carried out in a Flex Cycler (Analytic Jena AG, Jena, Germany) with 2.5 μl of purified DNA, 0.4 mM dNTP, 2 mM MgCl₂, and 1 U of Taq DNA polymerase (Invitrogen, Karlsruhe, Germany). The cycling protocol was hot start at 95°C for 3 min, then 95°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1 min for a total of 35 cycles, which was followed by 72°C for 5 min. The primers used were: ARCO (5’-CGTATGCTACGGTAGCATACAGTAAAGAATTGA-3’), SKIR (5’-GGGTATTCTGGACTGATCATG-3’), CRYP (5’-GGGTATTCTGGACTGATCATG-3’), CRYP1 (5’-TGCTTGACGCGGTAAGATGA-3’), and CRYP2 (5’-ACAACCTACGTCTCCTGC-3’). DNA amplicons for *Arcobacter* were run on a 1.5% agarose gel in Tris–borate–EDTA (Carl Roth, Karlsruhe, Germany) buffer at 100 V for 40 min. Resulting band sizes were 257 bp for CRYP1–CRYP2, 401 bp for BUTZ, and 641 bp for SKIR. The primers for species identification were chosen for the *Arcobacter* spp. A. butzleri, *A. cryaerophilus*, and *A. skirrowii*, which are found in poultry and are associated with human health risks.

**RESULTS**

*Arcobacter* was detected in all broiler flocks analyzed. Out of 135 broiler carcasses and parts, 58 (43.0%) samples were positive for *Arcobacter*. The prevalence within the flocks sampled was 16.0% (n = 25), 40.0% (n = 25), 42.9% (n = 35), 48.0% (n = 25), and 68% (n = 25) in flocks 4, 5, 1, 2, and 3, respectively. In parallel, the broiler flocks were examined for the presence of *Campylobacter*, and all flocks were positive for this bacterium, with rates of up to 100% (data not shown). Carcass contamination levels with *Arcobacter* were 48.0% (n = 25) in carcasses before evisceration, 48.0% (n = 25) in carcasses after evisceration, and 60.0% (n = 25) in carcasses after chilling. Cut poultry ready for packaging was positive with 40.0% (n = 30) in breasts and 20.0% (n = 30) in breast fillets.

In the analysis of turkey samples, it could be shown that the prevalence was lower (P < 0.05) when compared with broiler samples. Out of 170 samples, 31 (18.2%) were positive for *Arcobacter* spp. Prevalences in samples from the various turkey flocks were 8.8% (n = 34), 14.7% (n = 34), 20.5% (n = 44), 20.7% (n = 29), and 27.6% (n = 29) in flocks 4, 5, 1, 2, and 3, respectively.

*Arcobacter*-positive turkey skin samples were found with prevalences of 30.0% (n = 30), 30.0% (n = 30), 20.0% (n = 10), and 13.3% (n = 30) for skin pre-evisceration, post-evisceration, pre-chilling, and post-chilling, respectively. Cut turkey meat showed prevalences of 6.7% (n = 25), 12.0% (n = 25), 13.3% (n = 25), and 6.7% (n = 25) for wings, thighs, breast cuts, and medallions, respectively. The qualitative analysis gave counts in the range of 1.7 and 2.4 log CFU/cm² for turkey skin.

In 58 *Arcobacter* isolates from broilers and 27 isolates from turkey, typed by PCR, *A. butzleri* was prevalent with 25 (43.1%) and 10 (37.0%) isolates in broilers and turkeys, respectively. *A. skirrowii* (31.0% in broilers and 33.3% in turkeys) was next, and was followed by *A. cryaerophilus* (25.9% in broilers and 29.6% in turkeys).

In broilers, *A. butzleri* and *A. skirrowii* were predominant in flocks 1 and 3 to 5. *A. cryaerophilus* was found in flocks 1 and 2; while it was dominating in flock 2, it was present at lower levels in flock 1 when compared with the other species. In samples from turkey flocks, *A. cryaerophilus*, *A. skirrowii*, and *A. butzleri* were isolated from samples of flocks 1, 2, and 3. In contrast to this, *A. butzleri* was the only species isolated from flocks 4 and 5 (data not shown).

**DISCUSSION**

*Arcobacter* was found in samples from all flocks of broilers or turkeys examined in this study. The origin and source of *Arcobacter* and its introduction into the slaughter line is still unknown. *Arcobacter* can be found in other sources like surface water and sewage sludge (9, 18, 23, 27). Its ability to attach to water distribution pipes was shown by Assanta et al. (27). It may inhibit colonization by *Arcobacter*, but the housing of the birds, in particular the litter, may be contaminated with this bacterium, thus leading to surface contamination of the feathers during the rearing period. *Arcobacter* was found in the slaughter environment of a broiler slaughterhouse and on neck skin samples, but not in the intestinal contents of

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**References**

the same flock (14). The processing water in poultry processing plants is considered an important source in Arcobacter contamination of carcasses (10, 15).

Our results showed a prevalence rate of 43.0% in broilers, which is close to the prevalence reported by Son et al. (26) in a study from the United States, in which 55.1% of samples were Arcobacter positive. The results of turkey samples along the slaughter line were still lower, with 15.6% of samples Arcobacter positive. Arcobacter was found in poultry and especially in broiler processing at high rates. One hundred percent of carcass samples collected at slaughter after evisceration and analyzed by whole-carcass rinse were positive in a study from Denmark (4). Neck skin samples taken along the slaughter line showed similar prevalences in other studies, and the quantification also showed high numbers of Arcobacter at levels from as low as 10\(^1\) to >10\(^3\)/g of neck skin in broilers (15, 33). It should be considered that cut poultry ready for packaging was also examined in our study, which contributed to the lower overall prevalence rates of Arcobacter found compared with other European studies, in which only carcasses prior to chilling were analyzed (15, 33). When chicken meat alone was analyzed, prevalence rates even much lower (as low as 23%) were found (19), which corresponds to our results.

An increase of the prevalence of Arcobacter after chilling on broiler slaughtering was seen, whereas there was a continuous decrease of Arcobacter on skin samples during turkey processing. In the course of processing, a sharp decrease of Arcobacter prevalence was seen by Son et al. (26): where the highest prevalence was found prescalad (96.8%), it was decreased pre- (61.3%) and postchill (9.6%). During cutting, prevalence of Arcobacter-positive turkey meat was seen at a similar level. This may underscore the fact that the slaughter environment has a major impact on contamination of poultry by Arcobacter, making predictions about the levels of contamination at different production steps difficult.

The species identification for the three prominent types showed Arcobacter spp. A. butzleri, A. cryaerophilus, and A. skirrowii present. In the majority of positive samples, A. butzleri was found. This is consistent with other studies on Arcobacter in poultry at different production levels (10, 19, 26). A. cryaerophilus was also routinely found (4), whereas A. skirrowii seems to be more susceptible to the isolation methods carried out so far and might be underreported (13).

When comparing prevalences of Arcobacter in different studies, it has to be borne in mind that there still is no standard method for isolating Arcobacter. In contrast to reports about the susceptibility of A. skirrowii to the selective agents formulation in modified charcoal–cefoxiperoxide–deoxycholate agar and cefoperazone–amptherocin–teicoplanin in agar (3, 4), we could isolate all three species with those media. Therefore, in addition to regional differences, the method of isolation is also responsible for actual prevalence and species distribution. In conclusion, the prevalence of Arcobacter spp. was lower (P < 0.05) in turkeys than in broilers at slaughter.

REFERENCES


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