Research Note

Prevalence and Concentration of Arcobacter spp. on Australian Beef Carcasses

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MS 12-093; Received 24 February 2012/Accepted 12 April 2012

ABSTRACT

The International Commission on Microbiological Specifications for Foods (ICMSF) classified Arcobacter spp. as emerging pathogens in 2002. Arcobacter spp. have been isolated from numerous food products at retail and from animal carcasses and feces at slaughter. A survey was conducted to determine both the prevalence and concentration of Arcobacter spp. on prechill beef carcasses. Surface swab samples were collected from 130 beef carcasses at the end of processing, prior to chilling. The concentration of Arcobacter spp. was determined by a most-probable-number per square centimeter (3 by 3) method with a limit of detection of 0.12 CFU/cm². Of the 100 carcasses examined from export abattoirs, 20 (20.0%) were contaminated with Arcobacter spp., and 5 of these had quantifiable levels of contamination ranging from 0.12 to 0.31 CFU/cm². Of the 30 carcasses examined at a pet food abattoir, 25 (83.3%) were contaminated with Arcobacter spp., and 10 of these had quantifiable levels of contamination ranging from 0.12 to 0.95 CFU/cm². Three species of Arcobacter, A. butzleri, A. cryaerophilus, and A. skirrowii, were identified by PCR. Each of the species was present in an approximately equal ratio from export abattoirs. This study demonstrates that slaughter practices at export abattoirs are sufficient to maintain both low prevalence and low levels of contamination of beef carcasses with Arcobacter spp.

Arcobacter spp. were first placed in the same family as Campylobacter spp. by Vandamme and De Ley (17). Arcobacter spp. can be differentiated from Campylobacter spp. by the ability to grow at 15 to 25°C and to grow aerobically. Only four of the six currently described Arcobacter species have been isolated from humans and animals, A. butzleri, A. cryaerophilus, A. skirrowii, and A. cibarius (9, 19). Arcobacter organisms have been isolated from the feces or intestines of pigs, sheep, horses, dairy cows, and cattle (20, 21). Arcobacter spp. have been isolated from the feces of healthy cattle in Belgium at an average of 11%, including 8 of 276 animals with more than 10⁵ CFU/g feces. Three species of Arcobacter have been isolated from cattle feces (10, 12, 20), with an overall prevalence ranging from 1.1% (10) to 10% (20).

There have also been a number of studies that report the detection and isolation of Arcobacter spp. from retail meat samples. Arcobacter spp. have been isolated more frequently from poultry than from red meat (3, 22). In recent years Arcobacter spp. have been isolated from retail samples of beef, chicken, and pork meats (5, 14). Prevalence is highest amongst poultry samples, with 73% in Australian samples compared with 29 and 22% in Australian retail pork and beef, respectively (14). However, there are a limited number of studies that report on the levels of Arcobacter either in fecal samples or at points along the processing chain to retail. A recent study of Belgian beef carcasses demonstrated the level of this organism on both pre- and postchill carcasses, as well as on retail minced beef (5). The aim of this current study was to determine the prevalence and concentration of Arcobacter spp. on prechill Australian beef carcasses. The infective dose of Arcobacter is unknown, and elucidation of the effect of processing not only on the prevalence but also the level of contamination with Arcobacter will aid in further understanding the risk to food safety posed by this organism.

MATERIALS AND METHODS

Bacterial strains. Reference strains included A. butzleri (NCTC 12481), A. cryaerophilus (NCTC 11885), A. skirrowii (NCTC 12713), and Campylobacter jejuni (ATCC 33291).

Collection of carcass samples. To enable the potential worst-case scenario of Arcobacter survival during beef cattle slaughter, prechill carcass samples were collected following the protocols of the Australian Quarantine and Inspection Service export carcass microbiological sampling program, Escherichia coli and Salmonella monitoring program (ESAM) (1). Briefly, these protocols identify three sites for sampling, including the brisket, flank, and rump or butt, with 100-cm² areas being sampled at each site (total of 300 cm²). Alternate sides were tested using the same method to minimize any bias associated with particular sides. ESAM sampling was performed using cellulose acetate Speci-Sponges (Nasco, Fort Atkinson, WI) hydrated with 10 ml of Arcobacter...
selective broth (ASB; Oxoid, Basingtoke, UK). All samples were held on ice overnight before processing at the laboratory the next day. Upon return to the laboratory, 90 ml of Arcobacter selective broth was added (total volume 100 ml) and the sample mixed using a stomacher for 30 s. Sampling was conducted on five occasions from October to December, with 25 carcasses sampled on each of four visits. Approximately every third or fourth carcass was sampled. Abattoirs A (n = 25), B (n = 25), and C (n = 50) were export-registered facilities (all located in southeast Queensland), and abattoir D (n = 30) was a knackery located in central Queensland used to process animals for pet food. This enabled the inclusion of carcasses which might be expected to have high prevalence and high counts (knackery) and low prevalence and low counts (export plants). The animals slaughtered at abattoir D were originally targeted for slaughter at an export facility but due to unforeseen circumstances, these animals were redirected for slaughter at the domestic knackery.

**Detection and concentration.** A most-probable-number (MPN) assay comprising 3 tubes by 3 tubes (0.5 to 0.005 cm²) was set up from the mixed sample. Briefly, 1 ml of the mixed swab was added to tubes 1, 2, and 3. A 0.1-ml aliquot of the mixed sample was added to tubes 4, 5, and 6. A 0.1-ml aliquot of tubes 1, 2, and 3 was added to tubes 7, 8, and 9, respectively. All tubes and swabs were incubated aerobically at 30°C for 48 h. All swabs were streaked onto modified cefsulodin-irgasan-novobiocin agar (mCIN; Oxoid) and blood agar containing cefoperazone, amphotericin B, and teicoplanin (CAT) supplement (BAC; Oxoid). A boiled cell lysate was made from each MPN tube by centrifuging a 1-ml aliquot at 17,320 × g for 3 min and then removing the supernatant. The pellet was resuspended in 200 μl of sterile distilled water and boiled for 10 min and then centrifuged at 17,320 × g for 3 min. The supernatant was used as a DNA template for PCR which was conducted according to the method of Houf et al. (8) Any tubes that tested positive for Arcobacter spp. by PCR were then streaked onto mCIN and BAC. All plates were incubated at 30°C and examined after 24 and 48 h. All suspect colonies were purified and confirmed using PCR. An MPN tube was considered to be positive if a colony was confirmed. MPN values were calculated using MPN Calculator Build 22 by Mike Curiale (http://www.i2workout.com/mcuriale/mpn/index.html).

**RESULTS**

Arcobacter spp. were isolated from 20 of the 100 export samples tested (Table 1). Of these, only five samples were quantifiable using the MPN method (limit of detection, 0.12 CFU/cm²). One sample had a count of 0.31 CFU/cm², and four had counts of 0.12 CFU/cm². The carcasses slaughtered at the knackery were contaminated at a rate of 83.3% (25 of 30) (Table 1), of which 10 samples were quantifiable. Most samples had a count of 0.12 CFU/cm², two had a count of 0.31 CFU/cm², and one sample each had counts of 0.77 and 0.95 CFU/cm².

**Arcobacter butzleri** and **A. cryaerophilus** were isolated from both export and knackery samples and **A. skirrowii** from knackery samples only. The relative percentage of each species from the positive samples varied, with **A. cryaerophilus** isolated from 62% of export and 48% of knackery samples and **A. butzleri** isolated from 50 and 24% of positive samples from export and knackery abattoirs, respectively. **A. skirrowii** represented 64% of contaminated knackery carcasses. Some carcasses were contaminated with more than one species of Arcobacter.

**DISCUSSION**

The prevalence of Arcobacter spp. on retail samples of various meats has been widely reported. The prevalence on retail beef products varies, with low prevalence (0 to 9%) (4, 5, 11–13) found in retail beef by some studies while higher rates of 22 to 37% have been reported by others (2, 14, 15). It is unclear whether this is due to a real difference in prevalence or to differences in methodologies. Studies of retail beef can suffer from a lack of recognition of the possibility of cross-contamination during handling and preparation at retail. This possibility was highlighted in the study of Rivas et al. (14), with retail meat samples of four species, collected at a single establishment on a single day, all contaminated with the same Arcobacter clone. Alternatively, the route of contamination may be from the slaughtered animal via fecal or hide contamination onto the carcass. There are a number of studies that examined the fecal carriage of Arcobacter spp. in cattle (11, 20, 21). The contamination of beef carcasses with other pathogens, most notably E. coli O157, has been linked to both hide (6) and fecal (7) cross-contamination.

Although the prevalence of pathogens on beef carcasses is of interest, the importance of the concentration of pathogens, such as E. coli O157, and the role this plays in foodborne disease have been highlighted in risk assessment studies (16). There are few published studies on the numbers of Arcobacter spp. in animal feces and a single publication (5) that describes the enumeration of Arcobacter on beef carcasses. This study examined the concentration of Arcobacter spp. on carcasses prechill. The average of prechill counts in the De Smet study (2.59 log CFU/cm²) (5) was higher than that detected in this study (0.25 log CFU/cm²)
The detection limit of this study, using an MPN methodology, was 0.12 log CFU/cm². The limit of detection in the De Smet study (1.13 CFU/cm²) was almost 10-fold greater than that in this study. Hence, it is possible that some of the 67 positive carcasses in that study that did not have quantifiable levels might have been quantifiable by use of an MPN methodology.

While no postchill carcasses were examined in this study, the study by De Smet et al. (5) found a significant (P < 0.01) decrease in the prevalence of Arcobacter on bovine carcasses after 24 h of forced-air chilling. Similarly, a study by Van Driessche and Houf (18) found a reduction in prevalence on pig carcasses after chilling. As most Australian beef carcasses currently undergo approximately 18 to 24 h of forced-air chilling, similar reductions in the prevalence and concentration of Arcobacter spp. could be expected. Both the low prevalence before chilling and the low numbers present on carcasses suggest that beef carcasses are unlikely to be the main cause of contamination of ground beef at retail if the cold chain from abattoir to retail remains intact.

In this study, only A. butzleri and A. cryaerophilus were isolated from export samples, with almost equal prevalence from prechill carcasses. Similarly, in a Belgian study, only A. butzleri and A. cryaerophilus were isolated from prechill carcasses (5). Only A. butzleri was isolated from an Australian study of ground beef (14), with no evidence of the presence of other species. There are a number of reasons why the relative prevalence of each species may vary at prechill or retail. Different methodologies may allow some species to outcompete others. Alternatively, certain species may be better able to survive the chilling process, which may vary between abattoirs and between countries. A. butzleri, A. cryaerophilus, and A. skirrowi have all been isolated from cattle feces, although A. butzleri is the predominant species (10, 12, 20). Without further work on the precise routes of contamination of carcasses with Arcobacter spp., it is not possible to understand why the relative abundance of each species varies through the processing chain.

Carcasses slaughtered at the pet food knackery were contaminated at a much higher rate than those slaughtered at an export abattoir (83.3 and 20%, respectively). Due to the inaccessibility of a central Queensland export facility to process all animals on the day required, some were diverted to abattoir D (knackery) for processing on the same day. This enabled some assessment of differing slaughter practices. The difference in contamination rate is most likely due to the slaughter practices at export and those at knackery abattoirs leading to different contamination levels. The key difference was in the use of bed dressing at the knackery abattoir as opposed to line dressing at the export abattoir.

This study highlights the presence of Arcobacter spp. on beef carcasses at the end of processing, prior to chilling. With an expected decrease, as demonstrated by the study of De Smet et al. (5), in both prevalence and numbers of Arcobacter species on postchill carcasses, the potential threat from these organisms to human health could be considered low. Good manufacturing practice as conducted in export abattoirs is sufficient to minimize the risk of Arcobacter contaminating beef carcasses. Further work is necessary to fully elucidate the survival of different Arcobacter species throughout the chilling process and the potential routes of contamination throughout the slaughter practice.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the management and staff of the abattoirs for their assistance and cooperation during this study. The authors acknowledge funding from Meat and Livestock Australia and matching funds from the Commonwealth Scientific and Industrial Research Organisation.

REFERENCES