Letters in Applied Microbiology 2004, **38,** 339–344 doi:10.1111/j.1472-765X.2004.01494.x

Investigation of arcobacters in meat and faecal samples of clinically healthy cattle in Turkey

H. Öngör[1], B. Çetinkaya[1], M.N. Açik[1] and H.I. Atabay[2]

[1]Department of Microbiology, Faculty of Veterinary Medicine, University of Firat, Elazig, and [2]Department of Microbiology, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

2003/0993: received 3 November 2003, revised 25 December 2003 and accepted 22 January 2004

ABSTRACT

H. ÖNGÖR, B. ÇETINKAYA, M.N. AÇIK AND H.I. ATABAY. 2004.

Aims: To investigate the presence of *Arcobacter* spp. in minced beef meat ($n = 97$) and rectal faecal samples ($n = 200$) collected from cattle immediately after slaughter at a local abattoir in Turkey.

Methods and Results: Meat samples were examined using three different isolation procedures (CAT-supplemented media, de Boer arcobacter isolation method and membrane filtration method), but only one method (CAT-supplemented media) was employed for faecal samples. The isolated *Arcobacter* strains were identified by genus- and species-(multiplex) specific PCR assays. *Arcobacter* spp. were isolated from 5 and 9·5% of meat and faecal samples respectively. Although the only *Arcobacter* sp. found in meat samples was *Arcobacter butzleri*, all three pathogenic species – *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* – were detected in the rectal swabs. No *Arcobacter* was isolated when the de Boer method was used for minced meat samples but the same five meat samples were found positive for arcobacters when CAT-supplemented media and membrane filtration method were used.

Conclusions: The membrane filtration method was found to be superior to the CAT-supplemented media, because it led to a reduction in competing microflora. However, the necessity for one filter and medium for each sample makes this method somewhat expensive. The multiplex-PCR (m-PCR) assay shortened significantly the time required for the identification of *Arcobacter* spp. and also removed the possibility of false positive results due to other campylobacteria.

Significance and Impact of the Study: This study reports the isolation of *Arcobacter* spp. in cattle for the first time in Turkey. The m-PCR assay enables the identification and differentiation of all arcobacters simultaneously in one-step PCR.

Keywords: *Arcobacter*, cattle, faeces, identification, isolation, multiplex-PCR, meat.

INTRODUCTION

Arcobacters, which were first isolated from aborted bovine foetuses and later from porcine foetuses (Ellis *et al.* 1977, 1978), were formerly referred to as 'aerotolerant campylobacters' due to their phenotypic and genotypic similarities to the genus *Campylobacter* (Neill *et al.* 1979). However, these organisms can be differentiated from campylobacters by their ability to grow under aerobic conditions and at temperatures between 15 and 30°C, although they require a microaerobic atmosphere for primary isolation (Vandamme *et al.* 1991). The genus *Arcobacter* was proposed to encompass these 'aerotolerant campylobacters' by Vandamme *et al.* (1991). At present, the genus *Arcobacter* is composed of four species – *Arcobacter butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. nitrofigilis*. Apart from *A. nitrofigilis*, the other three species are associated with human and animal diseases (Vandamme *et al.* 1992a; Mansfield and Forsythe 2001; On *et al.* 2002). Arcobacters were recovered

Correspondence to: B. Çetinkaya, Department of Microbiology, Faculty of Veterinary Medicine, University of Firat, 23119 Elazig, Turkey (e-mail: bcetinkaya@firat.edu.tr).

from a wide variety of sources, from poultry carcasses to drinking water by various researchers (de Boer *et al.* 1996; Collins *et al.* 1996; Atabay *et al.* 1998; Rice *et al.* 1999; Wesley and Baetz 1999; Houf *et al.* 2001). In the case of cattle, arcobacters have been isolated from aborted foetuses, preputial sheath washing, mastitis and faeces of calves with diarrhoea (Ellis *et al.* 1977; Logan *et al.* 1982; Gill 1983; Wesley 1997).

The presence of this organism has also been reported in faeces of clinically healthy cattle with the prevalence figures ranging from 3·6 to 14·3% by several researchers (Wesley *et al.* 2000; Golla *et al.* 2002; Kabeya *et al.* 2003).

Arcobacter butzleri and *A. cryaerophilus* are also implicated as human pathogens, as these organisms have been isolated from clinical samples of humans with enteritis and bacteraemia (Tee *et al.* 1988; Taylor *et al.* 1991; Mansfield and Forsythe 2001). Risk factors for human infection include consumption of undercooked/precooked contaminated foods of animal origin (Corry and Atabay 2001).

The identification of *Arcobacter* spp. relies mainly upon conventional phenotypical tests (Vandamme *et al.* 1992b). Although a variety of isolation procedures have been employed, a standard method with general acceptance is not yet available. In addition, arcobacters are biochemically inert and have fastidious growth requirements, which make their speciation problematic using standard phenotypic procedures (On 1996). Therefore, more specific and rapid methods are required to overcome these problems. For this purpose, a number of PCR assays using species-specific primers have been developed and used with success (Harmon and Wesley 1996; Gonzalez *et al.* 2000; Houf *et al.* 2000).

The objective of this study was to investigate the presence of *Arcobacter* spp. in minced meat and faecal samples of clinically healthy cattle in Turkey using various isolation procedures with subsequent identification of the isolates by genus- and species-specific PCR assays.

MATERIALS AND METHODS

Sample collection

Minced meat samples from beef cattle were collected from 97 retail markets, which were well distributed in the eastern part of Turkey. In addition, a total number of 200 rectal swab samples of faeces were collected from cattle immediately after slaughter at a local abattoir. The swab samples were transferred to the laboratories within tubes containing 0·9% NaCl.

Methods of isolation of *Arcobacter*

Minced meat samples from beef. Three different methods were used for the isolation of *Arcobacter* spp. from meat samples. In the first method, 1 g of minced meat was aseptically inoculated into 10 ml Brucella broth (Difco, Detroit, MI, USA) with CAT supplement (cefoperazone, 8 mg l^{-1}; amphotericin, 10 mg l^{-1} and teicoplanin, 4 mg l^{-1}) (SR 174E, Oxoid, Basingstoke, UK) and the samples were incubated aerobically at 30°C for 48 h. These enriched samples were then plated onto Mueller-Hinton agar (CM337, Oxoid) supplemented with 5% (v/v) lysed horse blood and CAT selective supplement. The plates were also incubated aerobically at 30°C until *Arcobacter*-like colonies were detected or for up to 3 days.

In the second method, the samples were examined according to the method described by de Boer *et al.* (1996). Briefly, 1 g of minced meat was aseptically inoculated into 10 ml Brucella broth (Difco) supplemented with 5% (v/v) lysed horse blood and antibiotics (cefoperazone, 32 mg l^{-1}; piperacillin, 75 mg l^{-1}; trimethoprim, 20 mg l^{-1} and cycloheximide, 100 mg l^{-1}) as previously described (de Boer *et al.* 1996). The enrichment media were incubated aerobically at 24°C for 48 h. The enriched samples were then plated onto Mueller-Hinton agar (CM337, Oxoid) containing the antibiotics used for supplementing the broth in the second method, and were incubated at 24°C for up to 3 days, aerobically.

In the third method, the procedure described by Steele and McDermott (1984) was applied with minor modifications. A 100-μl aliquot of sample, enriched in CAT-supplemented Brucella broth as described in the first method, was dispensed using a micropipette onto 47 mm diameter 0·7 μm pore size cellulose acetate membrane filters (Millipore, Bedford, MA, USA) laid on the surface of a 5% sheep blood agar. When placing the 100-μl aliquot on the membrane, care was taken to avoid the inoculum spilling over the filter edge. The plates were incubated aerobically at 37°C for approx. 1 h before the filter was removed. After removing the filter, the fluid was spread evenly across the surface of the medium and the inoculated plates were incubated at 30°C for 48–72 h, aerobically.

Rectal swab samples of faeces. Faecal samples were examined using the same method as employed in the first method described above. The tubes containing the rectal swab samples were vortexed, and then 100 μl of each sample was transferred into CAT-supplemented enrichment broth and incubated aerobically at 30°C for 48 h.

Identification of the *Arcobacter* isolates using multiplex-PCR (m-PCR)

DNA extraction. A few representative colonies from cultures were suspended into an Eppendorf tube containing 300 μl distilled water. Each suspension was treated with

300 μl of TNES buffer (20 mmol l^{-1} Tris, pH 8·0; 150 mmol l^{-1} NaCl; 10 mmol l^{-1} EDTA, 0·2% SDS) and Proteinase K (200 μg ml^{-1}) and the suspension was kept for 2 h at 56°C. Following 10 min of boiling, the same amount of phenol (saturated with Tris–HCl) was added to the suspension. The suspension was shaken vigorously by hand for 5 min and then centrifuged at 11 600 *g* for 10 min. The upper phase was carefully transferred into another Eppendorf tube and 3 M sodium acetate (0·1 volume) and 95% ethanol (2·5 volumes) were added to the suspension, which was left at −20°C overnight to precipitate the DNA. The pellet, obtained following the centrifugation at high speed for 10 min, was washed twice with 95 and 70% ethanol, respectively, each step followed by 5 min centrifugation. Finally the pellet was dried and resuspended in 50 μl of distilled water.

m-PCR. The PCR was performed in a touchdown thermal cycler (Hybaid, Middlesex, UK) in a total reaction volume of 50 μl containing 5 μl of 10X PCR buffer (10 mmol l^{-1} Tris–HCl, pH 9·0, 50 mmol l^{-1} KCl, 0·1% Triton® X-100), 5 μl 25 mmol l^{-1} $MgCl_2$, 250 μmol l^{-1} of each deoxynucleotide triphosphate, 2 U of *Taq* DNA polymerase (MBI Fermentas, Hanover, MD, USA), 1 μmol l^{-1} of each primer (Iontek, Bursa, Turkey) and 5 μl of template DNA. A pair of primers derived from 16S rRNA (Harmon and Wesley 1997) was first used to identify arcobacters at genus level. Then, positive DNA samples were examined further using three pairs of primers (specific for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*), which were described by Houf *et al.* (2000) for differentiation at species level. Amplification procedures used for both genus- and species-specific PCR (m-PCR) were described previously (Harmon and Wesley 1997; Houf *et al.* 2000). In the genus-specific PCR, products with the molecular size of 1223 bp and in the m-PCR, the sizes of 257, 401, 641 bp, were considered indicative for identification as *Arcobacter* spp., *A. cryaerophilus*, *A. butzleri* and *A. skirrowii* respectively. The amplified and digested products were detected by ethidium bromide (0·5 μg ml^{-1}) staining after electrophoresis at 80 V for 1 h in 1·5% agarose gels.

Reference *A. butzleri* [LMG (Laboratorium voor Microbiologie en Microbielle Genetica, Ghent, Belgium) 10828] and *Campylobacter coli* [NCTC (National Collection of Type Cultures, London, UK) 11366] strains were included as positive and negative controls in all assays.

RESULTS

As summarized in Table 1, of the 97 minced meat samples examined, five (5%) were found to be positive for arcobacters. Nineteen (9·5%) of the 200 rectal swab samples were also found positive for *Arcobacter* spp. Amplification with the expected molecular size of 1223 bp was obtained in the examination of DNA samples extracted from the representative colonies of positive meat and faecal isolates by genus-specific PCR, confirming the identification of *Arcobacter* spp.

No *Arcobacter* was isolated when de Boer isolation method was used from minced meat samples. However, the same five meat samples were found positive for arcobacters when CAT-supplemented media and membrane filtration methods were employed.

In m-PCR, all five isolates recovered from minced meat were identified as *A. butzleri*. The distribution of species identified from faecal isolates was as follows: 7% (14/200) *A. butzleri*, 2% (4/200) *A. cryaerophilus* and 0·5% (1/200) *A. skirrowii* (Table 1).

DISCUSSION

Although the presence of *Arcobacter* has previously been shown in poultry products (Atabay *et al.* 2002a), this study reports the isolation of *Arcobacter* spp. in cattle for the first time in Turkey.

Various broths with distinctive supplements have been employed in the isolation of arcobacters so far, but none has been adopted as the standard (de Boer *et al.* 1996; Collins *et al.* 1996; Lammerding *et al.* 1996; Atabay and Corry 1998). A number of factors such as the type and concentrations of antimicrobial compounds in these media might influence the growth and isolation rate of *Arcobacter* (Atabay and Corry 1998; Atabay *et al.* 2002a). In the present study, attempts to isolate *Arcobacter* from meat samples by the de Boer method (de Boer *et al.* 1996) resulted in failure. The low sensitivity of this method has been noted previously (Houf *et al.* 2000; Ohlendorf and Murano 2002). In addition, this method has been reported to fail in the detection of *A. cryaerophilus*-positive samples (Houf *et al.* 2000). On the contrary, successful isolation was

Table 1 Distribution of *Arcobacter* spp. isolated from minced beef meat and rectal samples of faeces collected from cattle

Type of samples	No. of samples	*A. butzleri*-positive samples (%)	*A. cryaerophilus*-positive samples (%)	*A. skirrowii*-positive samples (%)	Total no. of positives (%)
Meat	97	5 (5)	–	–	5 (5)
Faeces	200	14 (7)	4 (2)	1 (0·5)	19 (9·5)

obtained by using the other two methods – CAT-supplemented media and a membrane filtration method. Several workers have found the filtration method as superior to the other isolation methods (Engberg *et al.* 2000; Atabay *et al.* 2002a). Although some workers experienced that CAT supplementation aided in the reduction of the growth of other types of bacteria (Gonzalez *et al.* 2000) heavy overgrowth by competitive microflora was still detected in CAT-supplemented media, which were employed for both meat and faecal samples in this study. The membrane filtration method was found to be superior to the CAT-supplemented media, because it lead to a reduction in competing microflora. However, the necessity for one filter and medium for each sample makes this method somewhat expensive. Large-scale studies are required to determine the best isolation protocols for detection of true prevalence, incidence and distribution of *Arcobacter* spp. from various materials of animal origin.

The isolation rate of arcobacters from meat samples was higher than 1·5 and 2·2%, which were reported for beef samples in the Netherlands and Japan respectively (de Boer *et al.* 1996; Kabeya *et al.* 2004). Various factors such as differences in sampling and isolation methods used in these studies may have contributed to this. However, the isolation rate was quite low when compared with the results of a recent study carried out in chicken products in Turkey, which reported rates of 95% from fresh chicken carcasses and 23% from frozen carcasses (Atabay *et al.* 2002a). This is not surprising because the findings of previous studies indicated that arcobacters were more prevalent in poultry meat than in red meat (de Boer *et al.* 1996; Kabeya *et al.* 2004). However, the isolation of the organisms from red meat samples, which were collected from retail markets appears significant when the risk for human health was considered.

All the meat isolates were identified as *A. butzleri* by using m-PCR described by Houf *et al.* (2000). This finding was in agreement with previous reports (Atabay *et al.* 2002a; Kabeya *et al.* 2004). The other species of *Arcobacter* (*A. cryaerophilus* and *A. skirrowii*), which were reported to be less frequent in meat samples including poultry meat (Houf *et al.* 2001; Atabay *et al.* 2002a; Kabeya *et al.* 2004) could not be detected in the meat samples of the present study.

The present study also investigated the presence of arcobacters in faeces of clinically healthy cattle and found that 9·5% of the animals carried *Arcobacter* spp., which was higher than the rate (3·6%) reported for Japanese cattle (Kabeya *et al.* 2003). When only *A. butzleri* isolates were taken into consideration, the isolation rate was calculated as 7·0%, which was relatively lower than the proportion (9·0%) reported for the presence of *A. butzleri* in beef cattle from the US (Golla *et al.* 2002). However, the prevalence of *Arcobacter* spp. was estimated to be much higher in dairy cows (Wesley *et al.* 2000; Golla *et al.* 2002). The differences between all these studies may be attributed to variations in sampling and isolation procedures, sample sizes and animal management practices. In any case, the excretion of *Arcobacter* through faeces in significant proportions is important as it is the main cause of environmental and carcass contamination.

The identification of arcobacters by conventional methods is time consuming, which requires at least 3–4 days and may not be reliable due to the fact that some arcobacters are biochemically inert and morphologically similar to campylobacters (Kiehlbauch *et al.* 1991; Johnson and Murano 1999). However, the development of the m-PCR assay in combination with primers specific to each *Arcobacter* species (Houf *et al.* 2000), which was employed in the present study, has shortened significantly the time required for the identification of arcobacters at the species level and also removed the possibility of false positive results due to campylobacters. The advantage of this assay over the other PCR assays reported by several workers (Harmon and Wesley 1997; Gonzalez *et al.* 2000; Winters and Slavik 2000) is that it enables the identification and discrimination of all *Arcobacter* spp. simultaneously in one PCR.

In conclusion, this study shows that arcobacters, which pose a threat for human health were present in meat and faecal samples of cattle that may play role in the contamination of the environment and human food chain. It is therefore believed that arcobacters deserve more attention as a food-borne illness in Turkey. Further research needs to be conducted to have a better understanding of the epidemiology of arcobacters in cattle.

REFERENCES

Atabay, H.I. and Corry, J.E. (1998) Evaluation of a new arcobacter enrichment medium and comparison with two media developed for enrichment of *Campylobacter* spp. *International Journal of Food Microbiology* **41**, 53–58.

Atabay, H.I., Corry, J.E. and On, S.L. (1998) Diversity and prevalence of *Arcobacter* spp. in broiler chickens. *Journal of Applied Microbiology* **84**, 1007–1016.

Atabay, H.I., Aydin, F., Houf, K., Sahin, M. and Vandamme, P. (2002a) The prevalence of *Arcobacter* spp. on chicken carcasses sold in retail markets in Turkey, and identification of the isolates using SDS-PAGE. *International Journal of Food Microbiology* **81**, 21–28.

de Boer, E., Tilburg, J.J., Woodward, D.L., Lior, H. and Johnson, W.M. (1996) A selective medium for the isolation of *Arcobacter* from meats. *Letters in Applied Microbiology* **23**, 64–66.

Collins, C.I., Wesley, I.V. and Murano, E.A. (1996) Detection of *Arcobacter* spp. in ground pork by modified plating methods. *Journal of Food Protection* **59**, 448–452.

Corry, J.E.L. and Atabay, H.I. (2001) Poultry as a source of *Campylobacter* and related organisms. *Journal of Applied Microbiology* **90**, 96–114.

Ellis, W.A., Neill, S.D., O'Brien, J.J., Ferguson, H.W. and Hanna, J. (1977) Isolation of *Spirillum/Vibrio*-like organisms from bovine foetuses. *The Veterinary Record* **100**, 451–452.

Ellis, W.A., Neill, S.D., O'Brien, J.J. and Hanna, J. (1978) Isolation of spirillum-like organisms from pig foetuses. *The Veterinary Record* **102**, 106.

Engberg, J., On, S.L., Harrington, C.S. and Gerner-Smidt, P. (2000) Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. *Journal of Clinical Microbiology* **38**, 286–291.

Gill, K.P.W. (1983) Aerotolerant *Campylobacter* strain isolated from a bovine preputial sheath washing. *The Veterinary Record* **112**, 459.

Golla, S.C., Murano, E.A., Johnson, L.G., Tipton, N.C., Cureington, E.A. and Savell, J.W. (2002) Determination of the occurrence of *Arcobacter butzleri* in beef and dairy cattle from Texas by various isolation methods. *Journal of Food Protection* **65**, 1849–1853.

Gonzalez, I., Garcia, T., Antolin, A., Hernandez, P.E. and Martin, R. (2000) Development of a combined PCR-culture technique for the rapid detection of *Arcobacter* spp. in chicken meat. *Letters in Applied Microbiology* **30**, 207–212.

Harmon, K.M. and Wesley, I.V. (1996) Identification of *Arcobacter* isolates by PCR. *Letters in Applied Microbiology* **23**, 241–244.

Harmon, K.M. and Wesley, I.V. (1997) Multiplex PCR for the identification of *Arcobacter* and differentiation of *Arcobacter butzleri* from other arcobacters. *Veterinary Microbiology* **58**, 215–227.

Houf, K., Tutenel, A., De Zutter, L., Van Hoof, J. and Vandamme, P. (2000) Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. *FEMS Microbiology Letters* **193**, 89–94.

Houf, K., Devriese, L.A., De Zutter, L., Van Hoof, J. and Vandamme, P. (2001) Development of a new protocol for the isolation and quantification of *Arcobacter* species from poultry. *International Journal of Food Microbiology* **71**, 189–196.

Johnson, L.G. and Murano, E.A. (1999) Comparison of three protocols for the isolation of *Arcobacter* from poultry. *Journal of Food Protection* **62**, 610–614.

Kabeya, H., Maruyama, S., Morita, Y., Kubo, M., Yamamoto, K., Arai, S., Izumi, T., Kobayashi, Y. *et al.* (2003) Distribution of *Arcobacter* species among livestock in Japan. *Veterinary Microbiology* **93**, 153–158.

Kabeya, H., Maruyama, S., Morita, Y., Ohsuga, T., Ozawa, S., Kobayashi, Y., Abe, M., Katsube, Y. *et al.* (2004) Prevalence of *Arcobacter* species in retail meats and antimicrobial susceptibility of the isolates in Japan. *International Journal of Food Microbiology* (in press).

Kiehlbauch, J.A., Plikaytis, B.D., Swaminathan, B., Cameron, D.N. and Wachsmuth, I.K. (1991) Restriction fragment length polymorphisms in the ribosomal genes for species identification and subtyping of aerotolerant *Campylobacter* species. *Journal of Clinical Microbiology* **29**, 1670–1676.

Lammerding, A.M., Harris, J.E., Lior, H., Woodward, D.E., Cole, L. and Muckle, C.A. (1996) Isolation method for recovery of *Arcobacter butzleri* from fresh poultry and poultry products. In *Campylobacter VIII. Proceeding of 8th International Workshop on Campylobacters, Helicobacters and Related Organisms* ed. Newell, D.G., Ketley, J. and Feldman, R.A. pp. 329–333. New York: Plenum Publishing Corporation.

Logan, E.F., Neill, S.D. and Mackie, D.P. (1982) Mastitis in dairy cows associated with an aerotolerant *Campylobacter*. *The Veterinary Record* **110**, 229–230.

Mansfield, L.P. and Forsythe, S.J. (2001) *Arcobacter butzleri* and *A. cryaerophilus* newly emerging human pathogens. *Reviews in Medical Microbiology* **11**, 161–170.

Neill, S.D., Ellis, W.A. and O'Brien, J.J. (1979) Designation of aerotolerant *Campylobacter*-like organisms from porcine and bovine abortions to the genus *Campylobacter*. *Research in Veterinary Science* **27**, 180–186.

Ohlendorf, D.S. and Murano, E.A. (2002) Prevalence of *Arcobacter* spp. in raw ground pork from several geographical regions according to various isolation methods. *Journal of Food Protection* **65**, 1700–1705.

On, S.L.W. (1996) Identification methods for campylobacters, helicobacters, and related organisms. *Clinical Microbiology Reviews* **9**, 405–422.

On, S.L., Jensen, T.K., Bille-Hansen, V., Jorsal, S.E. and Vandamme, P. (2002) Prevalence and diversity of *Arcobacter* spp. isolated from the internal organs of spontaneous porcine abortions in Denmark. *Veterinary Microbiology* **85**, 159–167.

Rice, E.W., Rodgers, M.R., Wesley, I.V., Johnson, C.H. and Tanner, S.A. (1999) Isolation of *Arcobacter butzleri* from ground water. *Letters in Applied Microbiology* **28**, 31–35.

Steele, T.W. and McDermott, S.N. (1984) The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. *Pathology* **16**, 263–265.

Taylor, D.N., Kiehlbauch, J.A., Tee, W., Pitarangsi, C. and Echeverria, P. (1991) Isolation of group 2 aerotolerant *Campylobacter* species from Thai children with diarrhea. *The Journal of Infectious Diseases* **163**, 1062–1067.

Tee, W., Baird, R., Dyall-Smith, M. and Dwyer, B. (1988) *Campylobacter cryaerophila* isolated from a human. *Journal of Clinical Microbiology* **26**, 2469–2473.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R. and DeLey, J. (1991) Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy emendation of generic descriptions and proposal of *Arcobacter* gen. nov. *International Journal of Systematic Bacteriology* **41**, 88–103.

Vandamme, P., Pugina, P., Benzi, G., Van Etterijck, R., Vlaes, L., Kersters, K., Butzler, J.P., Lior, H. *et al.* (1992a) Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. *Journal of Clinical Microbiology* **30**, 2335–2337.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., Van Den Borre, C. *et al.* (1992b) Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *International Journal of Systematic Bacteriology* **42**, 344–356.

Wesley, I.V. (1997) *Helicobacter* and *Arcobacter*: potential human foodborne pathogens? *Trends in Food Science and Technology* **8**, 293–299.

Wesley, I.V. and Baetz, A.L. (1999) Natural and experimental infections of *Arcobacter* in poultry. *Poultry Science* **78**, 536–545.

Wesley, I.V., Wells, S.J., Harmon, K.M., Green, A., Schroeder-Tucker, L., Glover, M. and Siddique, I. (2000) Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Applied Environmental Microbiology* **66**, 1994–2000.

Winters, D.K. and Slavik, M.F. (2000) Multiplex PCR detection of *Campylobacter jejuni* and *Arcobacter butzleri* in food products. *Molecular and Cellular Probes* **14**, 295–299.