Pet cats as carriers of Arcobacter spp. in Southern Italy

M.T. Fera¹, E. La Camera¹, M. Carbone¹, D. Malara² and M.G. Pennisi²

1 Department of Pathology and Experimental Microbiology, Faculty of Medicine, University of Messina, Messina, Italy
2 Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Messina, Messina, Italy

Keywords
Arcobacter spp., blood, lymph node, oral swab, PCR, pet cats.

Correspondence
Maria Teresa Fera, Dipartimento di Patologia e Microbiologia Sperimentale, Policlinico Universitario, Torre Biologica, 98125 Messina, Italy. E-mail: mfera@unime.it


doi:10.1111/j.1365-2672.2008.04133.x

Introduction

Arcobacter organisms were first isolated from bovine foetuses by Ellis et al. (1977). They were initially designated Campylobacter cryaerophilus based on their Campylobacter-like morphology, aerotolerance and growth at 25°C. In 1991, some of the campylobacters, referred to as aerotolerant campylobacters, were transferred into the new genus, Arcobacter (Vandamme et al. 1991). This genus is composed of potentially pathogenic species, Arcobacter butzleri, Arcobacter cryaerophilus, Arcobacter skirrowii, Arcobacter cibarius and of nonpathogenic species, Arcobacter nitrofigilis, Arcobacter halophilus and a number of not yet established species such as candidatus Arcobacter sulfidicus (McClung et al. 1983; Vandamme et al. 1991, 1992b; Wirsen et al. 2002; Donachie et al. 2005; Huf et al. 2005). The nonpathogenic species have been isolated from a variety of habitats, but no association has been reported from human or animal yet. The other species, A. butzleri, A. cryaerophilus, A. skirrowii and A. cibarius have been isolated from animal faeces and food of animal origin (Van Driessche et al. 2003, 2004; Rivas et al. 2004; Huf et al. 2005). Clinically healthy farm animals were also found to harbour Arcobacter as part of the normal intestinal flora (Van Driessche et al. 2005; Aydin et al. 2007). Arcobacter infections in humans have also been described (Phillips 2001). Arcobacter butzleri, A. cryaerophilus and A. skirrowii have been isolated mainly from stool specimens from patients with diarrhoea and in some cases septicaemia (Vandamme et al. 1992a; Lerner et al. 1994; Mansfield and Forsythe 2000; Woo et al. 2001; Vandenbergh et al. 2004; Wybo et al. 2004; Prouzet-Mauléon et al. 2006). Most infections are believed to result from the consumption of contaminated food and drinking water (Wesley 1996; Corry and Atabay 2001). This is consistent with high rates of arcobacter carriage in a variety of foods comprising poultry, pork and beef (Atabay et al. 2006; Aydin et al. 2007) although the role of other,
nonfood exposures in the epidemiology of arcobacters is still unknown. Arcobacters are also present in sewage, surface water, sea water, ground water and drinking water suggesting a ubiquitous distribution of these organisms in the environment (Jacob et al. 1993; Rice et al. 1999; Assanta et al. 2002; Moreno et al. 2003; Fera et al. 2004). The reported high prevalence of Arcobacter spp. in animal faecal samples (Van Driessche et al. 2003) could explain the presence of the organisms in water. Specifically, the transmission of arcobacters appears to be by a faecal-oral route through contaminated food and water or by direct contact with infected humans or with carrier animals (Vandamme et al. 1992a; Phillips 2001; Lehner et al. 2005; Van Driessche et al. 2005). Contamination of the environment by domestic and wild animal faeces could represent an alternative exposure pathway for human infection. Epidemiological studies on the presence of arcobacters in cats are limited (Petersen et al. 2007; Houf et al. 2008). As no data claiming the occurrence of arcobacters in cats were available in Italy, the aims of this study were to determine the carriage rate and the distribution of Arcobacter spp. in different biological samples (oral swabs, blood, fine needle lymph node aspirates) from 85 pet cats in Southern Italy, by using species-specific PCR assay and thus to elucidate their potential significance as sources of human Arcobacter infection. Moreover, the potential clinical significance of the infection in cats was also investigated by means of analysis of recorded clinical data.

**Materials and methods**

**Cats**

A total of 85 pet cats (39 males and 46 females) were enrolled in this study between November 2003 and June 2006. Data concerning age, breed, gender and neutering status, environmental history (indoor/outdoor, single-cat household/multi-cat household) of the cat, presence of flea and tick infestation, preventive anti-flea procedures were recorded, together with physical examination findings and laboratory investigations.

**Clinical samples**

Blood samples obtained by aseptic procedures from the jugular veins of cats were placed in ethylene-diamine-tetra-acetate treated tubes. Whole blood was stored at −20°C until molecular testing was performed. A lymph node fine needle aspirate and one oral swab from the vestibular area, gingiva or fauces were also taken for molecular analysis from each cat. Both samples were stored at −20°C before being tested.

DNA extraction

DNA was extracted from all materials using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s instructions. Briefly, clinical samples were added to an appropriate volume of phosphate-buffered saline and homogenized by vortexing. Twenty microlitres of a proteinase K solution (20 mg ml⁻¹) and 200 μl of buffer AL provided in the kit were then added, followed by incubation at 56°C for 10 min. Next, 200 μl of ethanol (96%) were added. The mixture was then loaded onto the QIAamp spin column and centrifuged at 6000 g for 1 min. The QIAamp spin column was placed in a 2-ml collection microtube, and the tube containing the mixture was discarded. The column material was washed (500 μl each) with the first washing buffer (buffer AW1) and with the second washing buffer (buffer AW2) provided in the kit. Finally, the DNA was eluted with 150 μl of a third buffer (buffer AE) provided in the kit.

**Oligonucleotide primers**

Three pairs of primers targeting the 16S and 23S genes were used in PCR assay for the detection and identification of Arcobacter spp. (Houf et al. 2000). These primers amplify a 257-bp fragment from *A. cryaerophilus* (ArcCRY), a 401-bp fragment from *A. butzleri* (ArcBUTZ) and a 641-bp fragment from *A. skirrowii* (ArcSKIR). The primers were synthesised by MWG-Biotech AG.

**PCR**

The amplification was performed by using a PCR Sprint Thermal Cycler (Hybaid, UK) and was carried out in 50 μl reaction volume containing 200 μmol l⁻¹ (each) dNTP, 0·1 μmol l⁻¹ (each) primer, 1× PCR buffer (50 mmol l⁻¹ KCl, 10 mmol l⁻¹ Tris–HCl pH 8·8, 0·1% Triton X-100), 50 mmol l⁻¹ MgCl₂, 2 U of Taq DNA polymerase and 5 μl of template DNA or water for the negative control.

The temperature profile for the PCR was as follows: an initial step of 15 min at 95°C, followed by a denaturation step for 1 min at 94°C, an annealing step for 1 min at 50°C and a primer extension step for 1 min at 72°C. After the 35th cycle, the extension step was prolonged for 10 min to complete synthesis of all strands and then the samples were kept at 4°C until analysis. A negative control lacking the DNA template was included in each experiment. The Arcobacter strains used as positive controls in the PCR tests included *A. butzleri* ATCC 49616, *A. cryaerophilus* ATCC 43157 and *A. skirrowii* ATCC 51132.
Detection of PCR products was performed by gel electrophoresis. Samples (5 μl) of final PCR products were loaded onto 1·5% agarose gel and subjected to electrophoresis in 1× TAE (0·04 mol l\(^{-1}\) Tris–acetate, 0·001 mol l\(^{-1}\) EDTA) buffer for 60–90 min at 100 V. The gels were stained with ethidium bromide and photographed under UV light trans-illumination. A 100-bp DNA ladder (BioLab New England) was included on each gel as a molecular size standard.

Sequence analysis

Four PCR-positive products, obtained from blood and lymph node aspirate samples and 10 randomly selected PCR-positive products, obtained from oral swab samples, were used for nucleotide sequencing. The amplicons were purified by using the Wizard SV Gel and PCR clean-up System (Promega) according to the manufacturer’s instructions. The sequencing was performed by Genelab (S. Maria di Galeria, Rome, Italy). Blast software (http://www.ncbi.nlm.nih.gov/blast/) was used to conduct homology searches of the GenBank database (Altschul et al. 1997).

Statistical analysis

Statistical analysis was performed using the software Graph-Pad IN-STAT for Windows and \(P < 0·05\) was used to indicate statistical significance. Comparison of medians of the age of *Arcobacter*-positive and *Arcobacter*-negative cats was performed with the Mann–Whitney test. Analysis of *Arcobacter* prevalence related to sex, clinical status, ectoparasite exposure, environmental history, was made with the Fisher’s exact test. Analysis of prevalence of *Arcobacter* PCR-positive results in cats affected by lymphadenomegaly and in cats with oral pathologies was made with the Fisher’s exact test. Comparison of results originating from the three different biological samples was made with the chi-squared test.

Results

When PCR positive results with any of the three different samples tested were considered as a whole, *Arcobacter*-specific DNA was found in 78·8% (67 of 85) of all the cats. Of 67 *Arcobacter*-positive cats, 66 (77·6%) and 29 (34·1%) were found positive for *A. butzleri* and *A. cryaerophilus*, respectively. Of the 29 samples positive for *A. cryaerophilus*, 28 were also found to be positive for *A. butzleri*. None of the examined samples gave PCR product for *A. skirrowii* (Table 1). *Arcobacter butzleri* was the most common species detected in the cats. As seen in Table 1, the *A. butzleri* detection rate was higher in oral swab samples than in blood and lymph node samples (76·5% vs 2·3%). One of the two cats that gave positive products for *Arcobacter* in blood and lymph node aspirate samples was found to show positive PCR results also in oral swab. The prevalence of *Arcobacter* positive cats was not significantly different between males (31 of 39; 79·5%) and females (36 of 46; 78·2%). *Arcobacter*-positive cats had a

### Table 1 *Arcobacter* species distribution among the *Arcobacter*-positive samples from both asymptomatic and symptomatic cats

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>No. of cats</th>
<th><em>Arcobacter</em> spp.</th>
<th>Positive results obtained in at least one sample (%)</th>
<th>No. Arcobacter PCR-positive products from each clinical sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ArcBUTZ</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>67 (78·8)*</td>
<td></td>
<td>2 (2·3)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>17</td>
<td>14 (82·3)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>68</td>
<td>53 (78·0)</td>
<td></td>
<td>2 (2·9)</td>
</tr>
<tr>
<td>Oral pathology</td>
<td>45</td>
<td>36 (80·0)</td>
<td></td>
<td>2 (4·4)</td>
</tr>
<tr>
<td>No oral pathology</td>
<td>40</td>
<td>31 (77·5)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Lymphadenomegaly</td>
<td>46</td>
<td>35 (76·0)</td>
<td></td>
<td>2 (4·3)</td>
</tr>
<tr>
<td>No lymphadenomegaly</td>
<td>39</td>
<td>32 (82·0)</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>


No *A. skirrowii* PCR-positive products were obtained from any clinical samples.

No *A. cryaerophilus* PCR-positive products were obtained from blood and lymph node aspirate samples.

*66 and 29 were found positive for *A. butzleri* and *A. cryaerophilus*, respectively.

†288 also positive for ArcBUTZ.

‡All also positive for ArcBUTZ.

§24 also positive for ArcBUTZ.

*17 also positive for ArcBUTZ.

**12 also positive for ArcBUTZ.
median age of 1-66 and the negative cats a median age of 4-00, but the difference was not very significant ($P = 0.0572$). Forty-four of 57 (77-2%) outdoor cats were Arcobacter-positive as were 33 of 28 (82-1%) indoor cats and the difference was not significant. Twenty-eight of 34 (82-3%) cats living in single cat household were Arcobacter-positive as were 39 of 51 (76-5%) living in a multi-cat household and the difference was not significant. The prevalence of Arcobacter DNA was slightly higher (43 of 53; 81-1%) in cats with fleas than in those not reported with fleas (24 of 32; 75%) and the difference was not significant.

The analysis of Arcobacter detection results in both asymptomatic and symptomatic cats is shown in Table 1. Arcobacter DNA was amplified from 14 of 17 healthy cats (82-3%) and from 53 of 68 cats with clinical signs (78%). The Arcobacter detection results according to clinical signs showed no significant difference. Of the 45 cats with oral pathologies, 36 (80%) were positive for Arcobacter spp., compared with 31 of 40 (77-5%) cats without oral pathologies. Of the 46 cats with lymphadenomegaly, 35 (76%) were positive for Arcobacter spp., compared with 32 of 39 (82%) cats without lymphadenomegaly.

PCR amplification of DNA obtained from two blood and lymph node samples, followed by DNA sequencing, resulted in the identification of A. butzleri in cats, with amplicons that had complete homology to the sequence for A. butzleri RM4018 (GenBank accession number CP000361.1). A comparison of the sequences of 10 randomly selected PCR products obtained from oral swab samples with known sequences in the GenBank database, showed that there was complete homology between our amplified products and the species A. butzleri (GenBank accession number CP000361.1, EF107749.1, AY621116, DQ464342.1, AF314538, DQ464340.1).

Discussion

This study represents the first investigation into the prevalence of Arcobacter spp. in pet cats in Italy assessed by using species-specific PCR assay. Overall, molecular analysis showed that the PCR scored positive with DNA Arcobacter spp. in 67 cats (78-8%). Comparing the results in this paper with those obtained in other animals such as dogs, pigs and cattle, the prevalence of Arcobacter spp. in this Italian cat group was very high (Van Driessche et al. 2003; Houf et al. 2008). Our results are consistent with those obtained in Belgium where the prevalence of arcobacters in porcine faecal samples on four unrelated farms was found to range from 59 to 85% (Van Driessche et al. 2004). However, it is difficult to compare the present findings with those reported in previous studies because of different diagnostic techniques and the different epidemiological approaches used. The prevalence of Arcobacter in animals has probably been underestimated because of inappropriate detection and cultural methods. Several antimicrobial agents used in selective media for the isolation of Arcobacter were found to be inhibitory to strains belonging to this genus (Houf et al. 2001). In a previous study, we revealed differences in the Arcobacter detection capabilities of PCR and culture methods with samples taken from a marine environment (Fera et al. 2004). In addition, we also demonstrated that A. butzleri can develop the viable but nonculturable (VBNC) state and survive in the environment for a long period (Fera et al. 2008). The VBNC state of Arcobacter spp. because of starvation and physical stress might influence the growth and isolation rate of Arcobacter. While the importance of VBNC cells in the initiation of human infection is not yet clear, it appears that cells in this state retain virulence, and should be considered involved in public health (Carlone et al. 2003; Houf and Stephan 2007).

There is a paucity of data regarding the occurrence of infections caused by Arcobacter spp. in cats. To our knowledge, only two studies have been published. Petersen et al. (2007) demonstrated that A. butzleri is harboured in the oral cavities of domestic pets by using a culture-independent PCR-DGGE technology. Houf et al. (2008) reported no isolation of arcobacters from cats by using cultural methods. In contrast, in the latter study arcobacters were isolated from faeces and oral swabs from dogs.

It is evident from the current study that arcobacters are harboured in the oral cavity of domestic cats. Two Arcobacter spp., A. butzleri and A. cryaerophilus, which are accepted as potentially pathogenic species for humans and animals were detected. A. skirrowii was not detected in this study. Consistently with most studies, we found that A. butzleri was the most frequently occurring species in all the samples examined (Van Driessche et al. 2003, 2004; Ongör et al. 2004; Aydin et al. 2007). However, other investigations have identified A. cryaerophilus as the dominant species in dogs, in cattle and in domestic geese, followed by A. skirrowii (Van Driessche et al. 2005; Atabay et al. 2008; Houf et al. 2008). The isolation technique used can influence the species of Arcobacter spp. which can be isolated (Van Driessche et al. 2005). This sustains the utility of molecular techniques for detection and identification of multiple Arcobacter species. This study reports for the first time cats concurrently infected with two Arcobacter species. Simultaneous occurrence of more than one species of Arcobacter has been reported for other animals such as pigs (Van Driessche et al. 2004) and cattle (Aydin et al. 2007). This contrasts with the findings in humans where only one genotype per person was isolated.
(Houf and Stephan 2007). The detection of different Arcobacter species may suggest multiple sources of contamination and infection.

In this study, there was no statistically significant association between the age, life style and clinical status of cats and their Arcobacter status. Our results are consistent with several other studies that reported the presence of arcobacters in clinically healthy animals (Van Driessche et al. 2003, 2004, 2005; Ongör et al. 2004; Atabay et al. 2008; Houf et al. 2008). Parallel findings documented the presence of arcobacters in healthy people (Vandenbergh et al. 2004; Houf and Stephan 2007). Petersen et al. (2007) identified A. butzleri in the saliva samples obtained from one cat and seven dogs with oral diseases but a pathogenic role of this bacterium cannot yet be maintained because of the lack of an extensive controlled clinical trials. Even though this study did not discover any clinical impact on cat health that could be attributed to Arcobacter infection, particularly in those affected by lymphadenomegaly and oral pathologies, no definite conclusion can be drawn. The zoonotic importance of oral colonization with arcobacters, as well as their importance in causing disease in cats, other animals and humans requires further studies.

Interestingly, Arcobacter bacteriaemia, as detected by PCR was found in two of 85 cats. The lymphadenomegaly in these cats could be related to bacteriaemia, because positive results were also obtained from lymph node aspirate samples. It will be important to investigate the factors resulting in bacteriaemia events in cats. Arcobacter spp. bacteriaemia in humans appears to be found almost exclusively in subjects with underlying disease and has been reported in a few cases. Lau et al. (2002) reported A. butzleri isolation from blood culture of a patient with acute gangrenous appendicitis. In another study, A. butzleri was recovered from blood culture in one patient with liver cirrhosis (Yan et al. 2000). Woo et al. (2001) reported A. cryaerophilus isolation from a traffic accident victim. The sources of the Arcobacter in the three cases were largely unknown.

In conclusion, the results of this study suggest that pet cats are carriers of Arcobacter spp. and the transmission of arcobacters from cats to humans by direct contact may be suspected to occur, because of the close interaction between pet cats and their owners. The high Arcobacter prevalence in oral cavity samples from cats could be consequent to the characteristic grooming behaviour of this animal that includes the perineal region and may sustain a faecal–oral contamination. Consequently, Arcobacter prevalence in feline faeces should also be evaluated to confirm that free roaming cats can contribute to the dissemination of this bacterium in the environment.

Acknowledgements

This study was supported by research grants (2004 financing) from the University of Messina, Italy. We thank Dr Leonarda Giacobbe for her contribution to the collection of samples.

References


Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper’s edge. Please remember that illegible mark-ups may delay publication.

Many thanks for your assistance.

<table>
<thead>
<tr>
<th>Query reference</th>
<th>Query</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AUTHOR: Please give manufacturer information for QIAamp spin column: company name, town, state (if USA), and country.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AUTHOR: Please provide city name and country for MWG-Biotech AG.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AUTHOR: Please provide city name for Hybaid.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AUTHOR: Please provide city name and country for BioLab New England.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AUTHOR: Please provide city name and country for Promega.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>AUTHOR: Please check this website address and confirm that it is correct (Please note that it is the responsibility of the author(s) to ensure that all URLs given in this article are correct and useable.).</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AUTHOR: Please give manufacturer information for Graph-Pad In-Stat: company name, town, state (if USA), and country.</td>
<td></td>
</tr>
</tbody>
</table>
Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

<table>
<thead>
<tr>
<th>Instruction to printer</th>
<th>Textual mark</th>
<th>Marginal mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leave unchanged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert in text the matter indicated in the margin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substitute character or substitute part of one or more word(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change to italics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change to capitals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change to small capitals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change to bold type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change to bold italic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change to lower case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change italic to upright type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change bold to non-bold type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert ‘superior’ character</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert ‘inferior’ character</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert full stop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert comma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert single quotation marks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert double quotation marks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert hyphen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start new paragraph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No new paragraph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transpose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close up</td>
<td>linking characters</td>
<td></td>
</tr>
</tbody>
</table>
| Insert or substitute space                                 | / through character or 
| between characters or words                                | \ where required                                 | 
| Reduce space between characters or words                   | between characters or words affected             | 

New matter followed by
\ or \(\)

(new character / or
new characters /

\ or \(\)

under character
e.g. \ or \(\)

over character
e.g. \(\)

\ or \(\) and/or

\ or \(\)

\ or \(\) and/or

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)

\ or \(\)