First detection of Arcobacter *sp. in Eurasian collared doves (*Streptopelia decaocto*)*

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Arcobacter,
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Summary
Data about the presence of *Arcobacter* in wild birds are currently lacking. In this study cloacal swabs from 95 collared doves (*Streptopelia decaocto*), submitted to the Department of Veterinary Medical Sciences (Bologna, Italy) between 2011 and 2013 from various urban and suburban areas of the Emilia-Romagna region (Northern Italy), were tested for the presence of *Arcobacter* sp. by a rRNA 23S nested Polymerase Chain Reaction (PCR). Eighteen out of 95 (19%) samples showed the expected PCR product. Further cultural and molecular studies are needed to assess the *Arcobacter* prevalence in wild birds and elucidate their potential epidemiological role as source of animal and human infections.

Prima evidenziazione di Arcobacter *sp. in tortore dal collare (*Streptopelia decaocto*)*

Parole chiave
Arcobacter,
Italia,
Polymerase Chain Reaction (PCR),
Streptopelia decaocto,
Tortora dal collare.

Riassunto
Nel presente studio sono riportati i risultati di un'indagine condotta su 95 tortore dal collare (*Streptopelia decaocto*) conferite al Dipartimento di Scienze Mediche Veterinarie dell'Università degli Studi di Bologna tra il 2011 e il 2013. Gli animali sono stati testati per la presenza di *Arcobacter* sp. Il DNA estratto da tamponi cloacali è stato analizzato mediante rRNA 23S nested PCR. Diciotto dei 95 campioni (19%) sono risultati positivi. Ulteriori indagini colturali e molecolari su più specie di volatili consentirebbero di approfondire la prevalenza dell'infezione da *Arcobacter* nei volatili selvatici e il loro potenziale ruolo epidemiologico nelle infezioni dell'uomo e degli animali.

The genus *Arcobacter* was proposed in 1991 (Vandamme *et al.* 1991), to accommodate organisms that displayed campylobacter-like motility and morphology, but differed from *Campylobacter* spp. for their aerotolerance and their ability to grow at lower temperatures.

At the present, the genus *Arcobacter* includes 18 species (Sasi Jyothsna *et al.* 2013). In animals, arcobacters have been associated to abortion, mastitis and gastrointestinal disorders, they have also been detected from clinically healthy farm animals (Collado and Figueras 2011). In humans, *Arcobacter butzleri, Arcobacter cryaerophilus,* and *Arcobacter skirrowii* have been isolated from stool samples of patients with gastroenteritis (Jiang *et al.* 2010, Patyal *et al.* 2011, Vandenberg *et al.* 2004, Wybo *et al.* 2004) and less frequently from faecal specimens of healthy humans (Houf and Stephan 2007, Vandenberg *et al.* 2004). *Arcobacter butzleri* has been classified as a serious hazard to human health[1] and as an important foodborne zoonotic pathogen (Cardoen *et al.* 2009). However, the incidence of *Arcobacter* spp. infections could be underestimated, because of inappropriate detection and inadequate identification procedures are available so far (Vandenberg *et al.* 2004).

Transmission of arcobacters to humans probably occurs through untreated drinking water,

[1] International Commission on Microbiological Specifications for Foods (ICMSF). 2002. Microorganisms in foods 7: Microbiological testing in food safety management. International Commission on Microbiological Specifications for Foods. Kluwer Academic/Plenum, New York, USA.

faecally contaminated as well as manipulation or consumption of contaminated raw and undercooked meat products (Lehner *et al.* 2005). The highest prevalence of *Arcobacter* spp. in foods of animal origin has been detected for poultry, followed by pork, beef and lamb (Rivas *et al.* 2004). A recent study reported *Arcobacter* spp. contamination in fresh vegetables (González and Ferrús 2011). At the same time, close contacts inter-humans (Vandamme *et al.* 1992, Vandamme *et al.* 2003) or with pets (Fera *et al.* 2009, Houf *et al.* 2008) are also potential source of *Arcobacter* spp. infection.

Data about the presence of *Arcobacter* spp. in wild birds are presently scanty (Collado and Figueras 2011). Eurasian collared dove (*Streptopelia decaocto*) is a Columbiformes species originally native to the India. It is capable of phenomenal range expansion and its spread and colonization are closely related to human activities (Romagosa and Labisky 2000). European invasion of collared doves from Asia began in the mid-20th century, extending from India to the Middle East, the Western Europe and, more recently, the Mediterranean countries such as Italy and Spain (Rocha-Camarero and Hidalgo De Trucios 2002, Sorace and Gustin 2008). Once they arrived, farmyards full of fallen grain and plentiful animal feed were the springboard for a rapid population expansion into rural, suburban and urban areas. This study aimed to evaluate the presence of *Arcobacter* in cloacal swabs of *S. decaocto*, in Italy.

Ninety-five adult collared doves (50 males, 45 females), submitted to the Department of Veterinary Medical Sciences (Bologna, Italy) between 2011 and 2013 from various urban and suburban areas of the Emilia-Romagna region (Northern Italy) and stored frozen, were tested for the presence of *Arcobacter*. DNA was extracted from cloacal swabs by a commercial kit (QIAamp DNA Stool Mini Kit, Qiagen, Hilden, Germany). A 23S rRNA nested-PCR was developed. The primary Polymerase Chain Reaction (PCR) amplifying a 383 bp fragment of 23S rRNA gene was performed using the following forward and reverse oligonucleotide primers: ARCO A (5′-TTCGGAGGAGATGGAGAA-3′) and ARCO B (5′-AGTTACGGCCGCCGTTTA-3′). Cycling conditions were as follows: 5 minutes of denaturation at 95° C and 35 cycles each consisting of denaturation at 94° C for 1 minute, annealing at 54° C for 1 minute and extension at 72° C for 1 minute. A final elongation step of 5 minutes at 72° C completed the reaction. In the secondary PCR amplifying a 316 bp fragment, primers ARCO 1 (5′-GTCGTGCCAAGAAAAGCCA-3′) and ARCO 2 (5′-TTCGCTTGCGCTGACAT-3′) (Bastyns *et al.* 1995), were used: 1 µl of product from the first PCR step was added to a final volume of 50 µl. PCR conditions were as described above, except for the annealing temperature (52 °C) and the number of cycles (n. 25). A distilled water negative control was included in both PCRs. The amplified products were visualized after electrophoresis in 1.5% agarose gel by ethidium bromide staining under UV light. The secondary PCR products were purified by using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and both strands were sequenced (Bio-Fab Research, Rome, Italy). The nucleotide sequences were compared with those available in Genbank by using the BLAST server from the National Center for Biotechnology Information[2]. Preliminary tests were performed on *A. butzleri* ATCC 49616^{T}, *A. cryaerophilus* ATCC 43158^{T} and *A. skirrowii* ATCC 51132^{T} reference strains.

Eighteen out of 95 (19%) samples showed the expected PCR product, identified as *Arcobacter* sp. by the sequencing. In view of the high density of collared doves in various environments (kindergartens, schools, farms, food companies), their faeces could represent a source of contamination to animals and humans. Workers in wildlife centres should also be informed about the potential risk of infection. The results of the present study should be considered preliminary to more comprehensive investigations taking in account that:

i. only 1 wild avian species has been considered;
ii. the samples examined were not suitable to microbial isolation attempts;
iii. as the samples were not collected from a focused sampling, the potential links between the positivity to *Arcobacter* in examined collared doves and the *Arcobacter* presence in origin geographical areas could not be evaluated.

Further cultural and molecular studies on larger number of samples from more species of birds living in rural and urban areas are needed to assess the *Arcobacter* prevalence in wild birds and elucidate their potential epidemiological role as source of animal and human infections.

[2] http://blast.ncbi.nlm.nih.gov/Blast.cgi.

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