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Short communication

Isolation of various *Arcobacter* species from domestic geese (*Anser anser*)

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Abstract

In this study, the prevalence and distribution of various *Arcobacter* spp. were investigated in samples taken from the cloacae of healthy domestic geese raised in Turkey. A membrane filtration technique with a non-selective blood agar was employed after enrichment in Arcobacter enrichment broth (AEB) to isolate a wide range of *Arcobacter* spp. In addition, the isolates were characterized phenotypically and identified at species level using a multiplex-PCR assay. A total of 90 cloacal swab samples taken from geese, collected on three farms (18, 25, 47 samples, respectively), were examined. Of the samples examined, 16 (18%) were found positive for *Arcobacter*. One *Arcobacter* species was isolated from each bird. Of the 16 *Arcobacter* isolates, 7 (44%), 7 (44%) and 2 (12.5%) were identified by m-PCR as *A. cryaerophilus*, *A. skirrowii* and *A. butzleri*, respectively. The present study indicates that domestic geese can harbour a variety of *Arcobacter* spp. in their cloacae. The presence of *Arcobacter* in geese may be of significance as reservoirs in their dissemination. Detailed research is needed for better understanding of the epidemiology and zoonotic potential of this emerging pathogen.

Keywords: *Arcobacter*; Geese; Poultry; Multiplex-PCR; Prevalence

1. Introduction

The genus *Arcobacter* that presently includes six species: *Arcobacter butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A nitrofigilis*, *A. cibarius* and *A. halophilus* was first proposed by Vandamme et al. (1991, 1992).

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Furthermore, an autotrophic, obligate microaerophile sulfide-oxidizing *Arcobacter* (*Candidatus Arcobacter sulfidicus*) has been described from coastal seawater (Wirsen et al., 2002). Another possible species, detected in aborted pig fetuses and ducks, awaiting for formal description has also been reported by On et al. (2003).

Arcobacter spp. were first isolated from aborted fetuses of livestock (Ellis et al., 1977). The organisms have also been associated with a range of other animal diseases such as reproductive disorders, mastitis and gastric ulcers (Logan et al., 1982; Suarez et al., 1997; de Oliveira et al., 1997). Clinically healthy farm animals were also found to harbour *Arcobacter* (Van Driessche et al., 2005; Aydin et al., 2007). *Arcobacter* spp. have also been associated with diarrhoea and occasionally septicemia in humans (Lastovica and Skirrow, 2000; Woo et al., 2001). *A. butzleri* is the species most often isolated from humans, but *A. cryaerophilus* and more recently *A. skirrowii* have also been associated with human diseases (Wybo et al., 2004; Prouzet-Mauleon et al., 2006).

Arcobacter spp. have been isolated from a variety of foods comprising poultry, pork and beef (Collins et al., 1996; de Boer et al., 1996; Atabay et al., 2006; Aydin et al., 2007) and water (Rice et al., 1999). Although arcobacters are commonly detected on poultry carcasses, different isolation rates were reported from live birds (Wesley and Baetz, 1999; Kabeya et al., 2003; Atabay et al., 2006). In some studies, no *Arcobacter* isolation was achieved from the intestines of chickens (Gude et al., 2005) but from 4 to 15% prevalance rate were reported from different studies conducted in the US (Wesley and Baetz, 1999), Japan (Kabeya et al., 2003) and Denmark (Atabay et al., 2006). Atabay et al. (2006) determined that of the chickens, turkeys and ducks examined in their study, ducks had the highest prevalence of the three poultry species examined. Thus, live birds are considered to have a significant role for the dissemination of *Arcobacter* spp. A recent study carried out by Dogan and Atabay (2006) demonstrated that domestic geese also harbour *Arcobacter* spp. However, in the latter study the *Arcobacter* spp. could not be identified to the species level. So far four *Arcobacter* spp., *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. cibarius*, have been isolated from chickens, ducks and turkeys (Kabeya et al., 2003; Atabay et al., 2006; Houf et al., 2005).

The current study was undertaken to determine the carriage rate and distribution of various *Arcobacter* spp. in domestic geese raised in Turkey.

2. Materials and methods

2.1. Samples from geese

A total of 90 cloacal swab samples taken individually from free range clinically healthy domestic geese (*Anser anser*), collected on three different farms (18, 25, 47 samples, respectively) in Kars, Turkey, were examined.

2.2. Isolation of Arcobacter by use of membrane filtration technique

This technique was previously used to isolate arcobacters from various sources. It depends on the ability of arcobacters, but not competitors, to pass through a membrane filter. Five or six drops (ca 100–120 μl) from enriched samples were inoculated onto a 47 mm diameter 0.45 μm pore size nitrocellulose membrane filter (HAWG047S1, Millipore, Billerica, MA, USA) placed on the surface of a non-selective blood agar plate as described earlier (Atabay and Corry, 1997).

2.3. Isolation media and method of examination

Arcobacter enrichment broth (AEB) was prepared in 10 ml quantities using arcobacter enrichment basal medium (Oxoid CM965) incorporating cefoperazone, amphotericin, teicoplanin (CAT) selective supplement (Oxoid SR174E) as described previously (Atabay and Corry, 1998). Blood agar comprised 5% (v/v) defibrinated sheep blood in blood agar base No. 2 (Oxoid CM271).

Sterile cotton-tipped swabs were employed to take samples from the cloacae of domestic geese (*Anser anser*). Each swab was moistened with AEB before the sample was taken from the cloaca and put into AEB (10 ml) immediately after the sample collection. The samples were transported to the laboratory. Each inoculated enrichment medium was agitated using a vortex mixer for approximately 1 min to release bacteria attached to swabs, and incubated microaer-

obically, which was achieved by using gas generating kits (Oxoid CN35, Hampshire, UK), with loose top at 30 °C for 48 h. After that, the enriched sample was plated onto a non-selective blood agar plate using the membrane filtration technique mentioned above. Incubation of the plates was also carried out microaerobically at 30 °C for up to 7 days. The plates were examined daily for any visible growth.

2.4. Phenotypic characterization of Arcobacter isolates

One suspect colony from each morphologically different type on each plate was checked by Gram stain and oxidase test. Two or three colonies per plate giving reactions typical for *Arcobacter* were purified by streaking on blood agar. Phenotypic characterization of the isolates was accomplished using the tests listed in Table 1. All tests were carried out according to previously recommended procedures (On et al., 1996; Atabay et al., 2006) and a reference strain of *A. butzleri* (DCC25) kindly provided by M. Waino and M. Madsen from Danish Institute for Food and Veterinary Research, Denmark, was included as positive control throughout the study.

2.5. Identification of the Arcobacter isolates using multiplex-PCR

In order to perform simultaneous identification of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, a mutiplex-PCR (m-PCR) assay was employed as described previously (Houf et al., 2000). Briefly, all isolates were grown on blood agar plates micoaerobically at 30 °C for 48 h and then, single colony from each isolate was used to extract bacterial DNA. The amplification was performed as described by Houf et al. (2000) and thermal cycles were performed in MJ Mini Cycler (BioRad, Hercules, CA, USA). Amplified products were size-separated by electrophoresis in 1.5% agarose. Gels were stained with ethidium bromide and a UV light used for visualization.

3. Results

3.1. Phenotypic characterization of Arcobacter isolates

A total of 16 strains of *Arcobacter* were isolated from the cloacae of 90 domestic geese in the present

Table 1
Phenotypic characteristics of *Arcobacter* species isolated from the cloacae of domestic geese raised in Turkey

Phenotypic tests used[a]	No. of isolates positive		
	Arcobacter cryaerophilus (7)	*A. skirrowii* (7)	*A. butzleri* (2)
Gram negative	7	7	2
Oxidase	7	7	2
Catalase	7[b]	7	2[c]
Urease	0	0	0
Alpha-haemolysis	0	0	0
Growth at			
Room temperature (O_2)	7	7[d]	2
30 °C (O_2)	7	7	2
30 °C (mO_2)	7	7	2
30 °C (AnO_2)	0	0	0
37 °C (mO_2)	0	2	2
42 °C (mO_2)	0	0	0
Growth on media containing			
2.0% NaCl	5	7	2
4.0% NaCl	4	7	0

O_2: aerobically; mO_2: microaerobically; AnO_2: anaerobically; NaCl: sodium chloride.
[a] All test plates were incubated microaerobically at 30 °C and were not otherwise stated.
[b] Two isolates were weakly positive.
[c] One isolate was weakly positive.
[d] One isolate grew weakly after 7 days of incubation.

Table 2
The carriage rates and species distribution of *Arcobacter* in the cloacae of free range domestic geese in Turkey

Origin of samples examined[a]	No. of samples positive			
	Arcobacter cryaerophilus	*A. skirrowii*	*A. butzleri*	Total number (%)
Kafkas University College Farm, Kars (18)[b]	4	1	1	6 (33)[c]
2nd Farm, Kars (25)	3	0	1	4 (16)
3rd Farm, Kars (47)	0	6	0	6 (13)
In total (90)	7	7	2	16 (18)

[a] The samples were obtained from different geographical locations in Kars province.
[b] Number of samples examined.
[c] The numbers in brackets represent percentages.

study. Only one *Arcobacter* species was isolated from each goose cloacal sample examined. *Arcobacter* spp. were differentiated from *Campylobacter* and related organisms by their ability to grow aerobically. All *Arcobacter* isolates were Gram negative and showed slightly curved rod and/or spiral shape under the microscope. All isolates were oxidase positive. Except for two strains of *A. cryaerophilus* and one of *A. butzleri* that showed weak catalase activity (production of bubbles after 10 s), all were strongly catalase positive (instant production of bubbles). All isolates, except for one strain of *A. skirrowii* that showed only traces of growth after 7 days of incubation, were able to grow aerobically at room temperature. In addition, all grew at 30 °C both aerobically and microaerobically, whereas none of the strains tested was able to grow anaerobically at 30 °C or microaerobically at 42 °C. Two strains of *A. butzleri* grew microaerobically at 37 °C but none of the *A. cryaerophilus* strains and only two of the seven *A. skirrowii* strains were able to grow at 37 °C under microaerobic conditions. Alpha-haemolysis and urease production were not observed for any strains. Apart from two strains of *A. cryaerophilus*, all *Arcobacter* strains were able to grow on media containing 2% NaCl, whereas three *A. cryaerophilus* and two *A. butzleri* strains did not grow in 4% NaCl.

3.2. Differentiation of Arcobacter isolates at species level by m-PCR

All *Arcobacter* strains isolated from domestic geese in the present study were identifed to the species level using m-PCR. A 257-bp, 401-bp and 641-bp fragment was observed for *A. cryaerophilus*, *A. butzleri* and *A. skirrowii*, respectively (Houf et al., 2000).

3.3. The prevalence and distribution of Arcobacter spp. in domestic geese

Of the 90 samples examined, 16 (18%) were found positive for *Arcobacter*. Of these strains, 7 (44%), 7 (44%) and 2 (12.5%) were identified as *A. cryaerophilus*, *A. skirrowii* and *A. butzleri*, respectively. As summarized in Table 2, of the 18 samples collected from the Kafkas University College Farm, six [33%; speciated as *A. cryaerophilus* (4), *A. skirrowii* (1) and *A. butzleri* (1)] were positive for *Arcobacter*. Of the 25 samples from the 2nd farm, 4 (16%) were positive for *Arcobacter* of which 3 were identified as *A. cryaerophilus* and 1 as *A. butzleri*. Of the 47 samples from the 3rd farm, 6 (13%) were positive for *Arcobacter*, all of which were identified as *A. skirrowii*.

4. Discussion

The prevalence of *Arcobacter* spp. was determined as 18% out of the 90 cloacal swab samples obtained from three different goose flocks in the current study. Since other parts of the goose intestine were not examined, we cannot comment on whether *Arcobacter* spp. exist in other sites of the intestine. Although only three goose flocks were analysed, a relatively high prevalence of *Arcobacter* was found in the present study in all three flocks. In a previous study in our laboratory, the prevalence of *Arcobacter* spp. were found to be 26% in goose cloacal swab samples

obtained from the same geographical region (Dogan and Atabay, 2006). In that study, a total of 100 goose samples obtained from four different flocks were examined and the isolates were identified to the genus level. The difference between the two studies may be due to a number of factors such as different sampling time(s) and inclusion of different flocks. It is considered that poultry species may be significant reservoirs of various *Arcobacter* spp. (Wesley and Baetz, 1999; Houf et al., 2005; Kabeya et al., 2003; Atabay et al., 2006; Gonzales et al., 2007). Birds, including ducks, turkeys and chickens were reported to harbour various *Arcobacter* spp. in their intestines (Ridsdale et al., 1998; Wesley and Baetz, 1999; Atabay et al., 2006). Isolation of *Arcobacter* spp. from the cloacae of clinically healthy domestic geese in the present and the previous study (Dogan and Atabay, 2006) suggest that the geese may also be natural carriers of these bacteria. In an earlier study from Denmark (Atabay et al., 2006) ducks were reported to have the highest carriage of *Arcobacter* spp. with a 75% prevalence rate at the flock level, while turkeys were found as the second with an 11% carriage rate and only 4.3% of the 70 chicken flocks examined were positive for *Arcobacter*. Ridsdale et al. (1998) also reported that one out of four duck flocks was positive for *Arcobacter* spp. A carriage rate of approximately 15% was reported from chicken cloacal swab samples in two independent studies (Wesley and Baetz, 1999; Kabeya et al., 2003). It appears from the gimdings of the current study that the prevalence of *Arcobacter* spp. in domestic geese may be lower than that of ducks and higher than those of turkeys and chickens.

The carriage rate of *Arcobacter* was found to vary depending on the goose flocks examined (33, 16 and 13% among three different flocks). This may be due to a number of factors such as breeding conditions and/or easy access to contaminated sources like water and/or other animals harbouring *Arcobacter*. Geese in the region where the samples were obtained are kept on small-scale family farms which also contain several other domestic animals wandering free range. Hence, they may get colonized from the surrounding environment and disseminate the organisms in their faeces to other contaminating sources such as water and other animals. Therefore geese could play a potential role in the transmission of *Arcobacter* spp. to humans and other animals since the geese are in very close contact with other animals and with family members in the region.

The findings of the present study demonstrate that various species of *Arcobacter* are found in domestic geese. Three *Arcobacter* spp., *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* which are accepted as the sole pathogenic species for humans and animals were detected in goose cloacae in this study. The dominant species were *A. cryaerophilus* and *A. skirrowii*, with *A. butzleri* the least prevalent species in the samples analysed (see also Table 2). These three *Arcobacter* spp. have also been detected in other poultry species (Ridsdale et al., 1998; Atabay et al., 2006). This indicates that poultry including geese can harbour a variety of *Arcobacter* species in their cloacae.

Interestingly, none of the *A. cryaerophilus* and five of the seven *A. skirrowii* strains isolated in this study were able to grow at 37 °C microaerobically. None of the *A. skirrowii* strains of this study showed alpha-haemolytic activity on blood agar. This is interesting since alpha-haemolytic activity is a distinctive characteristic of *A. skirrowii* although non-haemolytic strains of *A. skirrowii* are occasionally reported (On et al., 1996; Atabay et al., 2006).

In conclusion, the results of the current study suggest that domestic geese are naturally colonized with various species of *Arcobacter* and may play a role as reservoirs in their dissemination. The relatively high prevalence of *Arcobacter* spp. detected in a limited number of goose samples analysed in the present study may be of significance. More research is required to establish the true epidemiology and/or prevalence of *Arcobacter* spp. in geese and to determine the exact role of these poultry species for the transmission of *Arcobacter* spp. to humans and animals.

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