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Isolation of *Arcobacter* spp. from domestic ducks and geese and identification of the recovered isolates by using molecular method

Elif ÇELİK*, Aliye GÜLMEZ SAĞLAM, Özgür ÇELEBİ, Salih OTLU
Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

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Abstract: This study aimed to investigate the role of healthy domestic geese and ducks as *Arcobacter* carriers. A total of 599 samples, including 330 cloacal swab and 116 fecal samples from geese, and 153 cloacal swab samples from ducks raised on family farms were analysed. For this purpose, the membrane filtration method was applied. In this study, *Arcobacter* spp. were isolated at rates of 16.7% and 12.93% from cloacal swab and stool samples of geese, respectively, and 26.14% from cloacal swab samples of ducks. Obtained isolates were identified by multiplex PCR (m-PCR) as *A. cryaerophilus*, *A. butzleri*, and *A. skirrowii*. Seasonal analyses of *Arcobacter* spp. that were isolated from the examined samples were done for the months in which the samples were collected. While the highest rate of *Arcobacter* spp. in cloacal swab samples in geese was obtained in October, *Arcobacter* spp. was found in stool samples in July. The highest isolation rate for ducks was obtained in July as well. These isolation rates suggest that the stool may play an important role in the transmission and spread of arcobacters. Consequently, ducks and geese, which are reservoirs for arcobacters because they carry *Arcobacter* spp. in their digestive systems, play a considerable role in the transmission of arcobacters to other animals and to humans, thereby being vectors of infection.

Key words: *Arcobacter* spp., ducks, geese, m-PCR, prevalence

1. Introduction

Arcobacters, described at the end of the 1970s as spiral-shaped bacteria isolated from the aborted fetuses of sheep, cattle, and pigs, are gram-negative bacteria capable of growing microaerobically and aerobically. They are distinct from campylobacters because of different structural formations in their fatty acid profiles (1), together with their growth abilities with exposure to atmospheric oxygen after first being isolated and subjected to low temperatures, such as 15–30 °C (2,3). The genus *Arcobacter* is a member of the family *Campylobacteraceae* and the rRNA superfamily VI in the Epsilon division of Proteobacteria (4).

Currently, this genus is represented by 25 species from diverse environments (5), including the feces of various domestic and wild animals (6); products from and carcasses of poultry, especially chicken, turkey, and quail (7); vegetables (8); milk and milk products (9); wild hunting birds (10); seashells (11); drinking water (12); and clinical samples of humans and animals (13,14). These are bacteria that may pose a threat to veterinary and public health; thus, they have been gaining an increasing amount of attention in recent years (15). In particular, three species — *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, which are members of the genus *Arcobacter*, described as food-borne and water-borne microorganisms (15,16), are associated with various diseases in humans and animals (17,18,19). Infections caused by the *Arcobacter* species present as bacteremia, septicemia, and diarrhea in humans, and diarrhea and abortion, among other diseases, in animals (20). Various foods, including water contaminated with poultry and poultry products, are the main sources for the transmission of these microorganisms (21).

Livestock, such as cattle, sheep, and pigs, carry the *Arcobacter* species at different rates in their digestive tracts. Many studies have isolated *Arcobacter* at varying rates in poultry (22); however, in other studies, *Arcobacter* isolation was not achieved in samples of poultry such as geese, ducks, and chicken (23); of the studied samples, only 1.3% in Nigeria (24), 5% in Iran (6), 8% in India (25), and 30% in Chile (26) yielded *Arcobacter*. Studies suggest that poultry, such as geese and ducks, in which a higher rate of isolation of *Arcobacter* was achieved, have different ratios of these bacteria as flora members in their digestive systems (27); therefore, they are reservoirs for *Arcobacter* (13,28). Members of the genus *Arcobacter*, isolated mostly from samples from the types of poultry whose feces are

* Correspondence: elita_3609@hotmail.com

critical carrier agents in the contamination of water and the environment, have been reported as *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* (22).

The goal of the current study was to investigate the role of healthy domestic geese and ducks as *Arcobacter* carriers. The study also examined the relationship between isolation of these bacteria and the seasons. In particular, the study investigated their role in transmission to humans, other animals, and environmental sources.

2. Materials and methods

2.1. Standard strains

Arcobacter butzleri, *A. cryaerophilus*, and *A. skirrowii* reference strains that were kindly provided by Prof. Francis Megraud (Bacteriology Laboratory of Victor Sagalen Bordeaux Hospital, France) were used as positive controls throughout the study.

2.2. Samples

Three hundred and thirty cloacal swab and 116 feces samples collected from geese and 153 cloacal swab samples from ducks raised on family farms in Kars Province from October 2015 to July 2015 (Table 1) were used in the current study as material. A microbiological analysis of the samples was performed using culture methods and the m-PCR technique.

2.3. Isolation and identification of Arcobacter spp.

Cloacal swabs and fecal samples were put into 5 mL of arcobacter broth (Fluka, 59848) containing CAT selective supplement (cefoperazone, amphotericin B, teicoplanin) (Oxoid, SR0174) and delivered to the laboratory within 3 to 4 h. Tubes inoculated with fecal samples and cloacal swabs were incubated at 30 °C for 48 h under microaerobic conditions using an Anaerocult C kit (Merck, 1.16275) for pre-enrichment. After incubation, the membrane filtration method recommended by Atabay et al. (29) was performed for enriched samples. All plates were incubated at 30 °C for 2 to 7 days in a microaerobic atmosphere.

Phenotypic tests, such as Gram stain, motility examination, catalase, oxidase, and indoxyl acetate hydrolisation tests were carried out on the colonies grown on blood agar plates.

2.4. Extraction

DNA was extracted by modifying the boiling method reported by Dashti et al. (30) for species-level identifications by m-PCR of the obtained *Arcobacter* spp. isolates. For this purpose, a few colonies belonging to the isolates that were incubated at 30 °C under microaerobic conditions in blood agar were put into 0.2 mL Eppendorf tubes containing 100 µL Trishydroxymethylaminomethane-ethylenediamine-tetraacetic acid (Tris-EDTA) buffer and suspended. Following incubation at 99.9 °C for 10 min, the tubes were kept at + 4 °C for about 10 min and then centrifugated at 10,000 rpm for 10 min. The supernatants were used as templates.

2.5. m-PCR

The m-PCR method, described by Houf et al. (22) was applied for species-level identifications of *Arcobacter* spp. isolates. In this species-specific technique, five primer sets (ARCO, BUTZ, CRY1, CRY2, SKIR) targeting the 16S rRNA and 23S rRNA sequences were used. The expected amplicon sizes for *A. cryaerophilus*, *A. butzleri*, and *A. skirrowii* in the m-PCR were 257, 401, and 641 bp, respectively.

Table 1. Numbers, locations, and sampling times of collected cloacal swab and feces samples.

Sampling place	Animal species	Sample	Sampling time	Number of samples
Çakmak village	Goose	Cloacal swab	October 2015	55
Çakmak village	Goose	Feces	October 2015	21
Çakmak village	Duck	Cloacal swab	October 2015	19
Bardaklı village	Goose	Cloacal swab	January 2015	40
Bardaklı village	Goose	Feces	January 2015	20
Bardaklı village	Duck	Cloacal swab	January 2015	12
Çamçavuş village	Goose	Cloacal swab	April 2015	100
Çamçavuş village	Goose	Feces	April 2015	25
Çamçavuş village	Duck	Cloacal swab	April 2015	20
Geçit village	Goose	Cloacal swab	July 2015	135
Geçit village	Goose	Feces	July 2015	50
Geçit village	Duck	Cloacal swab	July 2015	102
Total				**599**

Thermal cycler conditions for each m-PCR reaction carried out for a total of 37 cycles were performed at 94 °C for 2 min (predenaturation), 94 °C for 45 s (denaturation), 61 °C for 45 s (annealing), 72 °C for 30 s (extension), and 72 °C for 10 min (last extension). m-PCR products were determined using 1.5% agarose gel. Electrophoresis was applied at 120 volts and 300 milliamperes for 25 min.

3. Results

3.1. Isolation results

In the current study, *Arcobacter* spp. were isolated from 330 cloacal swabs and 116 feces samples at a rate of 16.7% (55/330) and 12.93% (15/116), respectively, for geese, and 26.14% (40/153) from 153 cloacal swab samples from ducks (Table 2).

3.2. Seasonal evaluation results

To assess the distribution of *Arcobacter* spp. isolated from the cloacal swab and fecal specimens, samples were taken during one month of each season—October (fall), January (winter), April (spring), and July (summer). Therefore, evaluations were done according to months (Table 1).

Arcobacter spp. were isolated from 11 (20%) of 55 cloacal swab samples collected in October, 6 (15%) of 40 samples in January, 12 (12%) of 100 samples in April, and 26 (19.26%) of 135 samples in July. In the feces samples, while an isolation rate of 4.8% was obtained from the samples collected in October, no *Arcobacter* spp. isolation was obtained from samples collected in January. Isolation rates were 8% (2/25) and 24% (12/50) in April and July, respectively. For ducks, *Arcobacter* spp. isolation results were 5.26% (1/19) in October, 0% (0/12) in January, 20% (4/20) in April, and 34.31% (35/102) in July (Table 3).

3.3. Identification results

The results of the m-PCR are as follows. Out of 55 cloacal swab isolates, 41 (74.5%), 11 (20%), and 3 (5.5%) were identified as *A. cryaerophilus*, *A. butzleri*, and *A. skirrowii*, respectively, in geese. In addition, of the isolates obtained from the feces, 13 (86.7%) and 2 (13.33%) were identified as *A. cryaerophilus* and *A. butzleri*. Isolation rates for ducks were 85% (34/40) for *A. butzleri*, 5% (2/40) for *A. cryaerophilus*, and 10% (4/40) for *A. skirrowii* (Table 4, Figure 1).

4. Discussion

Poultry farming is very important because poultry products are the most consumed animal products. Most of the world's poultry meat comes from chickens, turkeys, ducks, geese, qualis, and ostriches. Regarding poultry meat consumption, chicken has the highest consumption rate at 70%, followed by turkey at 8%, and other avian animals at 22% (28). The high prevalence of cross contamination in poultry farms has been a reason for isolating arcobacters from poultry products (31). The presence of arcobacters in the feces of healthy domestic poultry supports this concern. Poultry, such as chickens, geese, and ducks, which are reported to carry arcobacters at different rates in their intestinal systems, play an important role in the contamination of water and the environment through their feces (13). For that reason, fecal samples (cloacal swab/

Table 2. Isolation rates (%) of *Arcobacter* spp. isolates obtained from cloacal swab and feces samples and their distribution by kind of sample.

Animal species	Sample	*Arcobacter* spp.	Total number of samples	%
Goose	Cloacal swab	55	330	16.7
Goose	Feces	15	116	12.93
Duck	Cloacal swab	40	153	26.14

Table 3. Isolation rates of *Arcobacter* spp. from cloacal swab and feces samples according to seasons.

Animal species	Sample	Seasons			
		Autumn (n,%)	Winter (n,%)	Spring (n,%)	Summer (n,%)
Goose	Cloacal swab	11/55 (20%)	6/40 (15%)	12/100 (12%)	26/135 (19.26%)
Goose	Feces	1/21 (4.8%)	0/20 (0%)	2/25 (8%)	12/50 (24%)
Duck	Cloacal swab	1/19 (5.26%)	0/12 (0%)	4/20 (20%)	35/102 (34.31%)

Table 4. Prevalence of *Arcobacter* species in cloacal swab and feces samples.

			Culture positive (n) (%)	PCR positive (n) (%)		
Animal species	Sample	Number of sample	*Arcobacter* spp.	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*
Goose	Cloacal swab	330	55 (16.7%)	11 (20%)	41 (74.5%)	3 (5.5%)
Goose	Feces	116	15 (12.93%)	2 (13.33%)	13 (86.7%)	0 (0%)
Duck	Cloacal swab	153	40 (26.14%)	34 (85%)	4 (10%)	2 (5%)

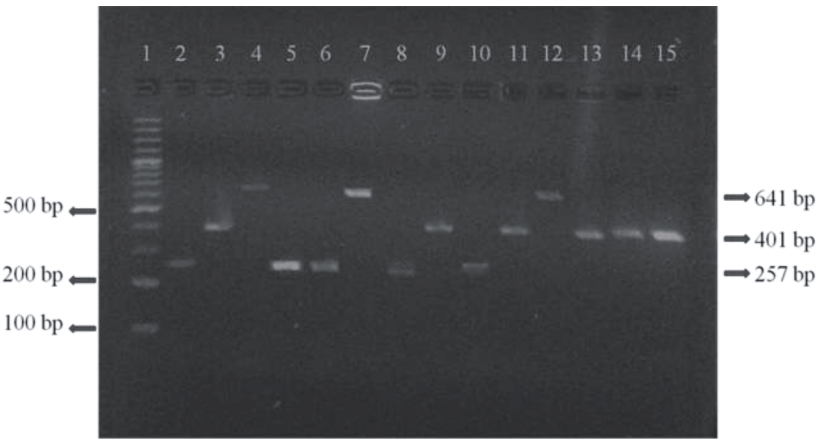

Figure. Gel electrophoresis image of m-PCR for *Arcobacter* species isolated from cloacal swab and feces samples of geese. 1: DNA marker (Gene ruler 100 bp DNA Ladder, Fermentas); 2: positive control, *A. cryaerophilus* (257 bp); 3: positive control, *A. butzleri* (401 bp); 4: positive control, *A. skirrowii* (641 bp); 5–13: isolates of cloacal swab; 14–15: isolates of feces.

feces), except those of animal products, are important research materials for understanding the transmission and presence of arcobacters.

In the current study, the isolation rate obtained was closer to the result obtained by Atabay et al. (32), 18% in cloacal swab samples. In the examined feces samples, however, a lower isolation rate than that of previous studies was obtained (33). While the *Arcobacter* isolation rate determined in cloacal swab samples of ducks was close to the results (40%) of Fernandez et al. (10), this rate was higher than that reported by Bogantes et al. (33) (5%). The differences in these isolation rates found in geese and ducks can be associated with the times the samples were taken, the nutritional status of the animals, and contact with contaminated sources, such as water or other creatures. As was concluded in previous studies (34), these animals are potential reservoirs for arcobacters because *Arcobacter* isolation was done in healthy geese as well. Another reason for the high isolation rates from geese and ducks may be that these animals were more associated with water than chickens and turkeys.

In this study, seasonal evaluations of cloacal swab samples taken from geese were interpreted according to the months of the year. Twenty percent (20%) of cloacal swab samples taken in October, 15% in January, 12% in April, and 19.26% in July tested positive for *Arcobacter* spp. In November, a 4.8% isolation rate was obtained with the feces samples, but *Arcobacter* spp. could not be isolated from any of the feces samples in January. While an 8% isolation rate was obtained in April, this rate was 24% in July. The maximum isolation in both the cloacal swab and feces samples was achieved in samples taken in the spring, fall, and summer months. These results are compatible with the results of previous research (35).

In the current study, which focused on the isolation rates of the *Arcobacter* species, the best isolation rates were obtained in the spring, fall, and summer months. For ducks, the highest isolation rate was obtained in the summer with a rate of 34.31%, followed by spring (20%), and fall (5.26%). No isolation rate was obtained for winter (0%). One reason could be that traditionally, animals are housed in confined spaces in winter months in Kars

Province. Geese and ducks in particular are only handled in winter months when they are breeding. With the start of spring and the mating season, geese and ducks leave the farms and thus, have more contact with the environment, especially with water and other animals.

The results of the current study suggest that geese and ducks carry *Arcobacter* spp. at varying rates. The study proved that they can be potential reservoirs for the transmission of these agents to humans, the environment, and other animals because healthy domestic geese and ducks harbor the *Arcobacter* species in their digestive systems. In Kars Province, raising geese is done by traditional methods that include housing the geese with other animals. This is an important issue, considering that arcobacters are associated with problems such as abortion, enteritis, and mastitis in domestic animals, as well as gastroenteritis, bacteremia, endocarditis, peritonitis, diarrhea, and septicemia in humans.

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