

UNIVERSITÀ DEGLI STUDI DI MILANO

# **ORIGINAL ARTICLE**



# Isolation of *Arcobacter* spp. and identification of isolates by multiplex PCR from various domestic poultry and wild avian species



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# Abstract

**Purpose:** The purpose of the present study was to determine the extent and seasonal prevalence of *Arcobacter* spp. in domestic poultry and wild birds in the Kars region of Turkey using multiplex polymerase chain reaction (m-PCR).

**Methods:** In this study, 1570 samples were collected from domestic poultry and wild avian species. The numbers of collected samples were as follows: 182 fecal samples from chickens, geese, and turkeys from family farms in the Kars region in Turkey; 1089 cloacal swab samples from chickens, geese, ducks, turkeys, and quails from family farms in this region; and 299 fecal samples from wild pigeons, crows, and owls in the same region.

**Results:** Arcobacter spp. were isolated from 17.43%, 35.77%, 3.63%, 6.87%, and 3.33% of the cloacal swab samples obtained from geese, ducks, chickens, turkeys, and quails, respectively. In the stool samples, *Arcobacter* spp. were isolated from 9.62%, 13.33%, and 4% of chicken, goose, and turkey samples, respectively. In wild birds, the isolation rates of *Arcobacter* spp. were 6.6%, 12.15%, and 0% in pigeons, crows, and owls, respectively. Using m-PCR, among 171 *Arcobacter* spp. isolates obtained from poultry and wild birds, 67, 78, 24, and 2 were identified as *Arcobacter cryaerophilus*, *Arcobacter butzleri*, *Arcobacter skirrowii*, and *Arcobacter cibarius*, respectively.

**Conclusions:** Both poultry and wild avian species exhibited variable rates of *Arcobacter* species positivity. The presence of *Arcobacter* spp. in the digestive tracts of healthy poultry and wild birds may serve as a potential reservoir for the dissemination of these microbes in the environment and their transmission to other animals and humans.

**Keywords:** Arcobacter spp., Cloacal swab, feces, Poultry, Wild bird, This research summarized from the doctoral thesis was supported as project number 2015-TS-10 by the Kafkas University Scientific and Technological Research Fund.

# Introduction

Arcobacter spp. are small  $(0.2-0.9 \times 0.5-3 \,\mu\text{m})$ , spiralshaped, and Gram-negative bacteria. They are non-spore forming and show corkscrew-like motility, aided by nonshielded polar flagella (Kayman 2012). In contrast to the genus *Campylobacter*, *Arcobacter* species are mostly aerotolerant and able to grow at temperatures below 30 °C (Brückner et al. 2020), although *Arcobacter*  *anaerophilus* is an obligate anaerobe in the genus (Sasi Jyothsna et al. 2013). Therefore, *Arcobacter* species are separated from campylobacters due to differences in their structural properties and fatty acid profiles (Gonulalan and Ertas Onmaz 2015), together with their ability to grow at temperatures of 15–30 °C and under aerobic conditions (Kayman 2012). The *Arcobacter* genus is a member of the *Campylobacteraceae* family and rRNA superfamily VI in the Epsilon division of *Proteobacteria* (Vandamme and De Ley 1991, On 2001).

To date, 28 Arcobacter species have been identified and characterized (Kim et al. 2019), which have been



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isolated from various hosts, foods, and environmental sources (Talay et al. 2016), such as sewage (Collado and Figueras 2011), streams and rivers (Laishram et al. 2016; Talay et al. 2016), drinking water, and municipal water (Ertas et al. 2010; Jalava et al. 2014). Arcobacter spp. have been isolated from various clinical samples from humans and animals (Petersen et al. 2007; Adesiji et al. 2011). They have been found in intestinal and fecal samples from a range of farm animals (Levican et al. 2013; Piva et al. 2013; Shakira et al. 2012); cloacal swabs and fecal samples from domestic poultry such as chickens, ducks, geese, and turkeys (Collado and Figueras 2011; Goni et al. 2016); and intestinal and fecal samples from numerous wild avian species (Fernández et al. 2007; Wesley and Schroeder-Tucker 2011; Di Francesco et al. 2014).

Within the genus, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* are the most common pathogenic species and are associated with various infections in humans and animals (Van den Abeele et al. 2014). These infections mostly appear clinically as abortion, enteritis, and mastitis in domestic animals (Patyal et al. 2011) and as gastroenteritis, bacteriemia, endocarditis, peritonitis, diarrhea, and septicemia in humans (Vandenberg et al. 2004; Samie et al. 2007). The primary potential transmission sources of these agents are various foods, especially poultry and products thereof, together with contaminated water (Shah et al. 2012).

Healthy domestic and wild avian species that harbor *Arcobacter* spp. in their digestive tracts serve as important hosts for these pathogens (Atabay et al. 1998; Van Driessche et al. 2003). Feces of avian *Arcobacter* reservoirs play a major role in the transmission of arcobacters to the environment, other animals, and humans. Therefore, both domestic poultry and wild birds are important in the carriage and spread of arcobacters (Di Francesco et al. 2014).

There have been no previous studies aimed at the isolation and identification of *Arcobacter* spp. in domestic poultry and wild birds in the Kars region in Turkey. The aim of the present study was to determine the extent and seasonal prevalence of *Arcobacter* spp. in domestic poultry (geese, chickens, ducks, turkeys, and quails) and wild birds (crows, pigeons, and owls) in the Kars region using the m-PCR protocol.

# **Material and methods**

# **Reference strains**

The Arcobacter butzleri (CIP 103493), A. cryaerophilus (CIP 104014), and A. skirrowii (CIP 103588) reference strains used during the stages of isolation and molecular identification were kindly provided by Prof. Francis Megraud (Bacteriology Laboratory of Victor Sagalen Bordeaux Hospital, France).

# Domestic poultry and wild bird samples

In this study, 1089 cloacal swab samples (chickens, n = 358; geese, n = 327; turkeys, n = 131; ducks, n = 123; quails, n = 150) and 182 fecal samples (chickens, n = 52; geese, n = 105; turkeys, n = 25) were obtained from domestic poultry raised on family farms in the Kars region from October 2013 to June 2015. Fecal samples were not collected from ducks or quails. In addition, 299 fresh fecal samples were collected from wild birds (pigeons, n = 167; crows, n = 107; owls, n = 25) in the same region from March 2011 to December 2015.

#### Isolation and identification of arcobacters

Cloacal swab and fresh fecal samples from domestic poultry and wild birds were placed in 5 ml of arcobacter selective broth (Fluka, 59848, India) containing cefoperazone, amphotericin B, and teicoplanin (CAT selective supplement) (Oxoid, SR174E, UK) and transferred to the microbiology laboratory within 3 to 4 h under cold chain conditions. Swabs were incubated at 30 °C for 48 h under microaerobic conditions using an Anaerocult C kit (Merck, 1.16275, Germany) for pre-enrichment. After incubation, the membrane filtration method recommended by Atabay and Corry (1997) 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 indoxyl acetate hydrolyzation, nitrate reduction, and  $H_2S$  reduction tests together with Gram staining, motility examination, and catalase, oxidase and urease tests were carried out on colonies grown on blood agar plates. Aerotolerance (25 °C and O<sub>2</sub>, 30 °C and O<sub>2</sub>-CO<sub>2</sub>, 37 °C and O<sub>2</sub>-CO<sub>2</sub>, 42 °C and CO<sub>2</sub>), growth in MacConkey agar (Oxoid, CM1169, England), and growth in the presence of 2–3.5% sodium chloride were evaluated. *Arcobacter* spp. isolates were kept at – 20 °C to be used for molecular identification.

## **DNA** extraction

A modified version of the boiling method of Dashti et al. (2009) was used for DNA extraction from suspected *Arcobacter* isolates. For this purpose, a few colonies belonging to the isolates incubated at 30 °C under microaerobic conditions in blood agar were maintained at 99.9 °C for 10 min in Tris-EDTA buffer (Sigma, 93283, Germany), after which the tubes were placed at +4 °C for 10 min and then centrifuged for 10 min at 10,000 rpm. The obtained supernatants were used as template DNA suspensions.

#### M-PCR

First, the m-PCR methods described by Houf et al. (2000) and Douidah et al. (2010) were applied for the species-level identification of *Arcobacter* spp. isolates. For these assays, a specific primer set (Arco, Skir, Butz,

Cry1 and Cry2) targeting 16S rRNA and 23S rRNA sequences under the m-PCR method described by Houf et al. (2000) was used. Then, the m-PCR method described by Douidah et al. (2010) was applied for the identification of the *A. cryaerophilus* isolates. In this m-PCR assay, species-specific primers for *A. butzleri*, *A. cibarius*, *A. cryaerophilus*, *A. skirrowii*, and *Arcobacter thereuis* (ButR, CibR, SkiR, TheR, ArcoF, CryF, CryR, GyrasF, GyrasR) were used.

For the m-PCR assay described by Houf et al. (2000), each PCR assay was carried out in a 20-µl volume consisting of 10 µl of Taq Master Mix (Qiagen, Cat No./ID: 201443), 20 pmol of primers (Arco, Skir, Butz, Cry1 and Cry2), 1.5 µl of DNase-free water, and 4 µl of template DNA. The thermal cycling conditions for each m-PCR assay were as follows: 94 °C for 2 min (predenaturation), followed by 35 cycles of 94 °C for 45 s (denaturation), 61 °C for 45 s (annealing), and 72 °C for 30 s (extension) and a final extension at 72 °C for 10 min. For the m-PCR assay described by Douidah et al. (2010), each PCR assay was carried out in a 25-µl volume consisting of 5 µl of Taq Master Mix (Qiagen, Cat No./ID: 201443), 50 pmol of primers (ButR, CibR, SkiR, TherR, ArcoF, CryF, CryR, GyrasF and GyrasR), 4.3 µl of DNase-free water, and 4 µl of template DNA. Prior to cycling, the samples were heated at 94 °C for 3 min. The PCR assay involved 30 cycles of denaturation (94 °C, 45 s), primer annealing (58 °C, 45 s), and chain extension (72 °C, 2 min). The amplified products were evaluated by electrophoresis in 1.5% agarose gels in 1X Tris-Boric Acid-EDTA buffer. The gels were run at 120 volts and 300 milliamperes for 25 min.

# Results

#### Isolation results

In this study, *Arcobacter* spp. were isolated at different rates in domestic poultry and wild birds. *Arcobacter* spp. were isolated from 121 (11.11%) out of 1089 cloacal swab samples and 20 (10.99%) out of 182 feces samples from domestic poultry. The rates of positive cloacal swab samples were 34.95% (43/123) for ducks, 16.21% (53/327) for geese, 6.87% (9/131) for turkeys, 3.33% (5/150) for quails, and 3.07% (11/358) for chickens. The positive rates for feces samples were 12.38% in geese, 9.62% in chickens, and 4% in turkeys. However, *A. butzleri* and *A. cryaerophilus* were isolated together from three goose cloacal swab samples and one duck sample.

In the analysis of fecal samples from wild birds, no *Arcobacter* spp. were isolated in the owl (n = 25) samples. The *Arcobacter* isolation rates in crows and pigeons were 12.15% (13/107) and 6.6% (11/167), respectively.

## Identification results

The m-PCR assays identified the isolates as *A. cryaero-philus* (395 bp, Douidah et al. 2010), *A. butzleri* (401 bp,

Houf et al. 2000), *A. skirrowii* (641 bp, Houf et al. 2000), and *A. cibarius* (1125 bp, Douidah et al. 2010), and the identification results are summarized in Table 1.

The m-PCR method defined by Houf et al. (2000) was used to identify the isolates obtained in the present study at the species level. As a result of this method, 84 *A. cryaerophilus*, 78 *A. butzleri*, and 7 *A. skirrowii* isolates were identified. Then, the m-PCR method proposed by Douidah et al. (2010) was applied to identify *A. cryaerophilus* isolates. As a result of this m-PCR method, 65 of the 84 *A. cryaerophilus* isolates were identified as *A. cryaerophilus* again, but 17 isolates were identified as *A. skirrowii* and 2 as *A. cibarius*.

#### Seasonal evaluation results

The isolation rates of Arcobacter spp. varied according to season and poultry type. In the cloacal swab samples from geese, the rate was highest in winter (23.53%), followed by summer (21.17%), autumn (18.52%), and spring (9.80%). In fecal samples from geese, the highest rate was observed in summer (20.75%), followed by spring (9.1%) and autumn (5%). No isolates were detected in winter. For ducks, sampling was performed only in spring and summer, and the isolation rate was higher in summer than in spring (37.74% and 33.33%, respectively). In the cloacal swab samples from chickens, the highest isolation percentage was detected in summer (6.12%), followed by winter (2.8%), and no isolation was carried out in autumn and spring. For turkeys, the bacterial isolation rate was highest in winter (17.39%), followed by summer (7.41%), and no Arcobacter isolates were detected in autumn and spring. In the fecal samples, isolation (10%) was carried out only in spring. For quails, sampling was performed only in autumn and winter periods. The isolation rate was 7.14% in autumn. Among wild birds, fecal samples were obtained from crows and pigeons only in summer and spring periods. The highest isolation rate was observed in summer (13.97% for crows and 7.4% for pigeons). The variation in the isolation rates of Arcobacter spp. according to the season and avian species are shown in Table 2.

## Discussion

The *Arcobacter* spp. isolation rate varied in both domestic poultry and wild birds. In cloacal swab samples, the highest isolation rate was found in ducks (35.77%), followed by geese (17.43%), turkeys (6.87%), quails (3.33%), and chickens (3.63%). The rates detected in fecal samples were 13.33%, 9.62%, and 4% in geese, chickens, and turkeys, respectively.

The isolation rate observed in cloacal swab samples from geese was similar to that reported by Atabay et al. (2008) (18%) and higher than that reported by Bogantes et al. (2015) (0%). The *Arcobacter* isolation rate detected

Avian species	Sample type	Number of sample	Number of samples isolated Arcobacter spp.	Number of isolated Arcobacter spp.	m-PCR positive (n, %)					
					A. cryaerophilus	A. butzleri	A. skirrowii	A. cibarius		
Goose	Cloacal swab	`327	53	57	25 (43.85%)	13 (22.81%)	17 (29.82%)	2 (3.51%)		
	Feces	105	13	13	11 (78.57%)	1 (7.14%)	1 (7.14%)	0 (0%)		
Duck	Cloacal swab	123	43	44	5 (11.36%)	36 (81.82%)	3 (6.82%)	0 (0%)		
Chicken	Cloacal swab	358	11	13	4 (30.77%)	6 (46.15%)	3 (23.08%)	0 (0%)		
	Feces	52	5	5	5 (100%)	0 (0%)	0 (0%)	0 (0%)		
Turkey	Cloacal swab	131	9	9	9 (100%)	0 (0%)	0 (0%)	0 (0%)		
	Feces	25	1	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)		
Quail	Cloacal swab	150	5	5	5 (100%)	0 (0%)	0 (0%)	0 (0%)		
Crow	Cloacal swab	107	13	13	1 (7.69%)	12 (92.31%)	0 (0%)	0 (0%)		
Wild pigeon	Cloacal swab	167	11	11	1 (9.09%)	10 (90.91%)	0 (0%)	0 (0%)		
Owl	Feces	25	0	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)		

**Table 1** Distribution of *Arcobacter* species identified at the result of m-PCR performed in cloacal swab and feces samples of domestic poultry and wild birds

in duck cloacal swab samples in the present study (35.77%) was very close to that reported by Fernández et al. (2007) (40%), whereas it was much higher than that found by Bogantes et al. (2015) (5%) and Silha et al. (2015) (4.2%). In contrast, A. cibarius was identified in two goose cloacal swab samples by m-PCR. The differences in the isolation rates in geese and ducks may be attributed to the sampling times, feeding conditions, contact with other animals, and access to contaminated water. Nevertheless, compared with those in other domestic poultry species, the Arcobacter isolation rate was highest in samples from geese and ducks. This finding is in accordance with those of other studies (Atabay et al. 2006; Gonzalez et al. 2007) that have concluded that geese and ducks are potential reservoirs of arcobacters. However, these discrepancies may reflect different methodological aspects, such as the amount of sample analyzed, type of culture medium, incubation atmosphere, and even the geographic area where sampling was performed.

In the present study, the *Arcobacter* isolation rate obtained from cloacal swab samples from chickens (3.63%) was lower than the rate found by Van Driessche and Houf (2007) (10%). Ho et al. (2008) detected no *Arcobacter* isolates in chicken cloacal swab samples. The findings of the present study on the *Arcobacter* isolation rate from chicken fecal samples (9.62%) were compatible with those found by Atabay et al. (2006) (4.3%) and Mohan et al. (2014) (8%). They were lower than those reported by Ho et al. (2008) (16.7%), Bogantes et al. (2015) (16%), and Fernandez et al. (2015) (30%). Previous studies that have examined samples of chicken intestinal contents have reported differences in isolation rates ranging between 0% and 100% (Van Driessche and Houf 2007; Ho et al. 2008).

In this study, the *Arcobacter* isolation rates in turkey cloacal swabs and fecal samples were 6.87% and 4%,

<b>2</b> Seasonal i	solation rate	(n, %) (	of Arco	<i>bacter</i> spp.	from c	loaca	l swał	o and	feces	samp	les of	domestic	poultr	y and	wild	birc	IS
•	<b>2</b> Seasonal i	<b>2</b> Seasonal isolation rate	2 Seasonal isolation rate (n, %) c	2 Seasonal isolation rate (n, %) of Arco	2 Seasonal isolation rate (n, %) of Arcobacter spp.	2 Seasonal isolation rate (n, %) of Arcobacter spp. from c	2 Seasonal isolation rate (n, %) of Arcobacter spp. from cloacal	2 Seasonal isolation rate (n, %) of Arcobacter spp. from cloacal swak	<b>2</b> Seasonal isolation rate ( <i>n</i> , %) of <i>Arcobacter</i> spp. from cloacal swab and	<b>2</b> Seasonal isolation rate ( <i>n</i> , %) of <i>Arcobacter</i> spp. from cloacal swab and feces	<b>2</b> Seasonal isolation rate ( <i>n</i> , %) of <i>Arcobacter</i> spp. from cloacal swab and feces samp	<b>2</b> Seasonal isolation rate ( <i>n</i> , %) of <i>Arcobacter</i> spp. from cloacal swab and feces samples of	<b>2</b> Seasonal isolation rate ( <i>n</i> , %) of <i>Arcobacter</i> spp. from cloacal swab and feces samples of domestic	<b>2</b> Seasonal isolation rate ( $n$ , %) of Arcobacter spp. from cloacal swab and feces samples of domestic poultry	<b>2</b> Seasonal isolation rate ( $n$ , %) of Arcobacter spp. from cloacal swab and feces samples of domestic poultry and	<b>2</b> Seasonal isolation rate ( $n$ , %) of <i>Arcobacter</i> spp. from cloacal swab and feces samples of domestic poultry and wild	<b>2</b> Seasonal isolation rate ( $n$ , %) of Arcobacter spp. from cloacal swab and feces samples of domestic poultry and wild bird

Avian species	Sample type	Seasons								
		Autumn ( <i>n</i> ) (%)	Winter ( <i>n</i> ) (%)	Spring ( <i>n</i> ) (%)	Summer ( <i>n</i> ) (%)					
Goose	Cloacal swab	10/54 (18.52%)	8/34 (23.53%)	10/102 (9.80%)	29/137 (21.17%)					
	Feces	1/20 (5%)	0/10 (0%)	2/22 (9.1%)	11/53 (20.75%)					
Duck	Cloacal swab	0/5 (0%)	ND	4/12 (33.33%)	40/106 (37.74%)					
Chicken	Cloacal swab	0/18 (0%)	4/44 (2.8%)	0/49 (0%)	9/147 (6.12%)					
	Feces	0/10 (0%)	0/10 (0%)	0/10 (0%)	5/22 (22.73%)					
Turkey	Cloacal swab	0/6 (0%)	4/23 (17.39%)	1/48 (2.1%)	4/54 (7.41%)					
	Feces	0/5 (0%)	0/5 (0%)	1/10 (10%)	0/5 (0%)					
Quail	Cloacal swab	5/70 (7.14%)	0/80 (0%)	ND	ND					
Crow	Cloacal swab	ND	ND	0/14 (0%)	13/93 (13.97%)					
Wild pigeon	Cloacal swab	ND	ND	0/18 (0%)	11/149 (7.4%)					
Owl	Feces	ND	0/25 (0%)	ND	ND					

respectively. These rates were higher than those found by Andersen et al. (2007) (2%), and the isolation rate in fecal samples was lower than that reported by Fernández et al. (2007) (28.6%).

Only a few studies have examined the presence of Arcobacter spp. in quails (Ok Anadut and Gumussoy 2005; Rahimi 2014). In the present study, the Arcobacter isolation rate in quails was 3.33%. The different isolation rates in samples obtained from various kinds of poultry may depend on geographic- and region-specific characteristics, farming styles, the isolation and identification methods used, sample collection, and transportation conditions and proximity to other animals. In the present study, Arcobacter isolation rates were considerably lower in quails than in ducks and geese (i.e., aquatic poultry). As noted in previous studies (Wesley and Miller 2010; Fernandez et al. 2015), this finding supports the hypothesis that arcobacters are not permanent commensals of the intestinal flora of poultry because of the high body temperatures of these animals (40.5–42 °C).

In this study, the identification of Arcobacter isolates using two m-PCR assays in domestic poultry was performed, and the isolates were initially identified as A. cryaerophilus, followed by A. butzleri and A. skirrowii. Similar to the findings of other studies (Rahimi et al. 2012), in this study, the most commonly isolated species in all the samples was initially A. cryaerophilus and were subsequently A. butzleri and A. skirrowii. In accordance with the findings of previous studies (Rahimi et al. 2012), the results of species-specific m-PCR revealed that the most common species in the samples from wild birds was A. butzleri, followed by A. cryaerophilus and A. skirrowii. Only a few studies have detected the presence of A. skirrowii in wild birds (Van Driessche and Houf 2007; Adesiji et al. 2011). In the present study, A. skirrowii was isolated and identified, albeit at a low rate, in ducks and chickens compared to geese. The distribution of Arcobacter spp. in different organisms may change depending upon the relationships of the hosts with other organisms and their environment as well as the ecological features and environmental conditions to which they are adapted.

In the present study, the samples were first assessed according to the presence of *A. cryaerophilus, A. but-zleri,* and *A. skirrowii.* For this purpose, the m-PCR method described by Houf et al. (2000) was used for identification of the obtained *Arcobacter* isolate sat the species level. Nevertheless, according to data obtained from other studies, while this m-PCR method presents the advantage of 100% reliable identification of *A. butzleri,* it produces false-positive identification results for *A. cryaerophilus* (Levican and Figueras 2013). Therefore, an m-PCR assay developed by Douidah et al. (2010) that detects 5 *Arcobacter* species (*A. butzleri, A. cryaerophilus, A.* 

*skirrowii*, and *A. cibarius and A. thereius*) was used. This m-PCR assay was preferred in the second step in the current study. The confirmation of the *A. cryaerophilus* isolates identified in the first m-PCR assay was thus performed, and other species that might be in the samples were then identified. Nevertheless, it is thought that different assays are necessary for the correct identification of these species and other potential pathogenic *Arcobacter* spp.

Although many studies have examined the presence of arcobacters in domestic poultry (Bogantes et al. 2015; Hassan 2017), only a few such studies have included wild birds (Di Francesco et al. 2014; Giacometti et al. 2015). In a study in Italy where cloacal swab samples were taken from 95 Eurasian collared doves (Streptopelia decaocto), Arcobacter spp. were detected in 18 (19%) samples by nested PCR amplification of the 16S rRNA gene (Di Francesco et al. 2014). However, Giacometti et al. (2015) detected no Arcobacter spp. in a study of fecal samples from 47 wild pigeons (Columbia livia). In a study in Chile of fecal samples from 60 pelicans and 60 sparrows, Arcobacter spp. were isolated at a rate of 13.3% in pelicans and 6.7% in sparrows, and the obtained species was identified as A. butzleri (Fernández et al. 2007). In the present study, the analysis of fecal samples from wild birds detected no Arcobacter spp. isolates in samples from owls (n = 25). The isolation rates in crows and pigeons were 12.15% (13/107) and 6.6% (11/167), respectively. The differences among isolation rates may be derived from different avian species or their association with water.

In the present study, according to the results of the m-PCR assay, 10 wild pigeon isolates were identified as A. butzleri, and one isolate was identified as A. cryaerophilus, while 12 crow isolates were identified as A. butzleri, and one was identified as A. cryaerophilus. The bacterial isolation rate in wild pigeons in the present study was lower than that reported by Di Francesco et al. (2014) (19%). However, Di Francesco et al. (2014) utilized the direct PCR technique to identify Arcobacter spp. in cloacal swab samples from Eurasian collared doves, whereas the present study employed culture methods. There are difficulties in detecting Arcobacter spp. via culture methods. It was not possible to compare the findings regarding Arcobacter isolation rates in wild birds obtained in the present study with those in the literature due to the limited number of studies involving Arcobacter isolation and identification in wild birds (Di Francesco et al. 2014; Giacometti et al. 2015). However, the discovery of Arcobacter isolates in wild avian species in the Kars region suggests that they may play an important role in transferring arcobacters to humans and other birds or animals via the contamination of the environment and water

In this study, it was not possible to compare the seasonal distribution of *Arcobacter* isolation rates across all four seasons, as samples could not be obtained from the studied poultry in each season. However, where seasonal isolation rates in domestic poultry could be evaluated, the highest values were found in winter for geese, in summer for ducks, summer for chickens, in winter for turkeys, and in autumn for quails. Therefore, the seasonal distribution of arcobacters in domestic poultry seems to vary. Such variation may be associated with the bird's age, growth stage, and type of poultry farming. Therefore, more extensive studies are needed to shed light on the seasonal distribution of *Arcobacter* isolation rates.

In wild birds (wild pigeon and crow), the seasonal distribution of *Arcobacter* could be examined only in spring and summer. No *Arcobacter* spp. were isolated from fecal samples collected in spring months, while *Arcobacter* was isolated from samples collected in summer.

#### Conclusion

The present study revealed that most domestic poultry (goose, duck, chicken, turkey, and quail) and some wild birds (crow and pigeon) carried *Arcobacter*, with varying bacterial isolation rates. By harboring *Arcobacter* species in their digestive systems, these birds may serve as potential reservoirs for the dissemination of *Arcobacter* species in the environment and their transmission to other birds, animals, and humans. Duck, goose, and chicken farming in the Kars region are mainly based on traditional practices, in which birds are housed with other farm animals. This may give rise to a significant risk of *Arcobacter* infections in domestic animals and humans in the region.

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Research involving human participitants and/or animals  $\ensuremath{\mathsf{N/A}}$ 

#### Informed consent

N/A

#### Authors' contributions

The authors read and approved the final manuscript.

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#### Ethics approval and consent to participate

The research was carried out in accordance with the report numbered 2014-005 of the Faculty of Veterinary Medicine Experimental Animals Ethics Committee of the Kafkas University.

#### **Competing interests**

The authors declare that they have no conflict of interest.

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