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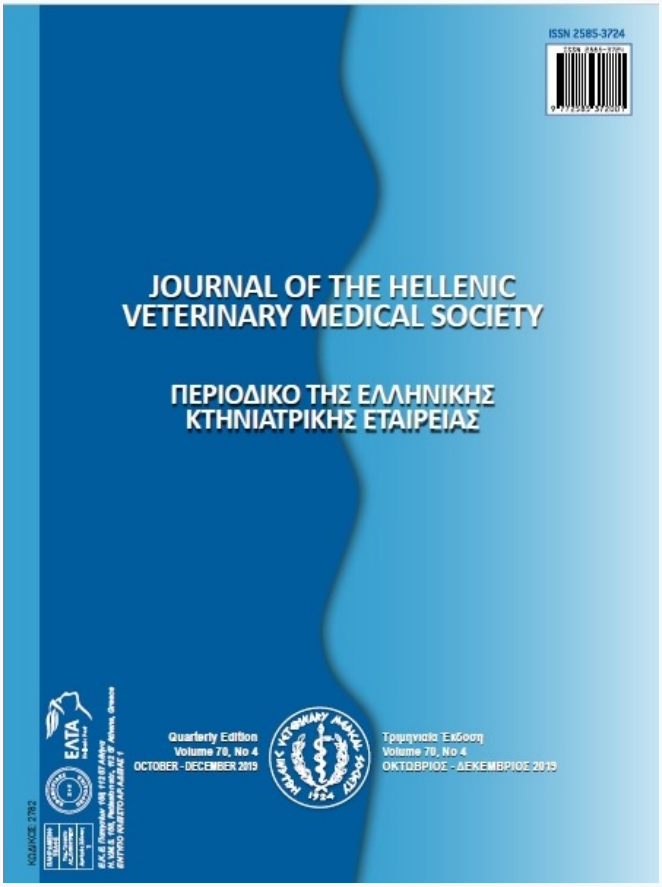

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S. SEIFI, R. KHOSHBAKHT, A. AZIZPOUR

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Research article
Ερευνητικό άρθρο

Occurrence of *Campylobacter*, *Salmonella*, and *Arcobacter* in pet birds of northern Iran

S. Seifi[1], R. Khoshbakht[1], A. Azizpour[2]

[1]*Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran*

[2] *Meshginshahr Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran*

ABSTRACT. Pet birds can harbor human pathogens and contribute to the transmission of infectious agents to human. Since many people are interested in keeping pet birds, this study was conducted in pet birds from Mazandaran province, northern Iran. Totally, 174 fecal samples of pet birds (cockatiel, canary, lovebird, parrot, mynah, goldfinch, budgerigar, macaw, dove, pigeon, and bulbul) were collected with sterile cotton swabs and submitted to Faculty of Veterinary Medicine, Department of Pathobiology (Amol, Iran). After extraction of total DNA, the samples subjected to molecular detection of the Campylobacter, *Salmonella*, and *Arcobacter* using polymerase chain reaction. A total of 114 (65.5%), 28 (16%), and 86 (49.4%) samples were found positive for *Campylobacter*, *Salmonella*, and *Arcobacter*, respectively. Furthermore, some birds showed contamination with two or all three of these bacteria. Results showed that mentioned bacteria can be detected from the apparently healthy pet birds. Therefore pet birds can be considered as potential carriers of these enteropathogens.

Keywords: Pet birds, Enterophatogens, PCR, Fecal samples

Corresponding Author:
Saeed Seifi, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, 24th Aftab, Imam Khomeini Street, Amol, Iran
E-mail address: s.seifi@ausmt.ac.ir

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INTRODUCTION

Infectious diarrhea is a major concern for public health worldwide and it caused by contamination of water and food with pathogenic bacteria, viruses, or parasites. Diarrheal disease is the primary cause of morbidity and mortality among children in developing countries (Kotloff et al., 2013). The common bacteria agents causing diarrhea are *E.coli*, *Salmonella*, *Campylobacter* and with less importance *Arcobacter* (Neupane et al., 2017). Pet birds have an important role as potential vectors of disease, especially regarding human health. People especially children and the elderly interests to keep pet birds, thus there is potential ability to transmit mentioned pathogens to humans.

In recent years, an increased number of human infections with bacteria of the *Campylobacter* type are observed (Grzybowska-Chlebowczyk et al., 2013). Worldwide, campylobacteriosis is the most commonly reported enteric bacterial infection in the human population in developed countries (Lyngstad et al., 2008). Although poultry products are important transmission vehicles to humans, the bacterium is common in pet birds, which live in close contact with humans (Griekspoor et al., 2013). Birds are ideal hosts for campylobacters, due to their relatively high body temperature (42 °C), also, the occurrence of *Campylobacter* spp. in the gut of apparently healthy birds has frequently been reported (Lillehaug et al., 2005). Transmission to humans can occur by aerosols, direct or indirect contact (Belén et al., 2010).

Salmonellosis is an important zoonotic infection seen in all species of animals. A variety of *Salmonella* species have been found in both apparently healthy and obviously diseased birds (Benskin et al., 2009). Transmission to humans was reported in different cases. Salmonellae in humans can cause enteric fever (typhoid) resulting from bacterial invasion of the bloodstream, and acute gastroenteritis resulting from food-borne infection/intoxication (Boseret et al., 2013).

Arcobacter spp. have been considered as potential zoonotic foodborne and waterborne agents and can be found in meat (veal, beef, pork and poultry), milk and water (Lehner et al., 2005). Infection in human patients causes diarrhea, abdominal pain and other symptoms including nausea, vomiting and fever. No association of *Arcobacter* with pathologies in poultry has been reported (Vandenberg et al., 2004).

Understanding the spread of bacterial pathogens in pet birds may help as a useful model for examining the spread of other disease organisms, both amongst birds, and from birds to other species. Thus, the aim of this study was to molecular identification of *Campylobacter*, *Salmonella*, and *Arcobacter* from pet or companion birds.

MATERIALS AND METHODS

Sampling: From July to November 2017, 174 fecal swab samples were collected from different species of the companion birds. Fantail pigeon (*Columba livia*) were the most represented species with 19.5% of the samples, followed by Budgerigar (*Melopsittacus undulatus*) with 11.5%, White-eared bulbul (*Pycnonotus leucotis*) 10.3%, Goldfinch (*Carduelis carduelis*) 9.1%, Cockatiel (*Nymphicus hollandicus*) 8%, Common mynah (*Acridotheres tristis*) 6.8%, and Rock pigeon (*Columba livia*) 5.7%. Other bird species composed less than 30% of the samples. All samples placed in separate sterile plastic bags to prevent spills and cross contamination and immediately transported to the laboratory in a cooler with ice packs. All birds were apparently in healthy condition and none received any antimicrobial treatment during the study period.

DNA extraction and PCR: The cotton swabs were placed in 1.5-ml tubes in 300 µl peptone water and vortexed thoroughly. Fifty microliters of each fecal suspension was used as input for the DNA extraction procedure. DNA extraction was done using stool DNA extraction kit (Bioneer, Daejeon, South Korea) according to the manufacturer recommendations with some modifications. Briefly, 100 mg of each pooled sample was mixed with 20 µl proteinase K and incubated for 10 min at 55 °C. After centrifugation of the mixture at 13000 rpm, the supernatant was mixed with 200 µl binding solution in a new tube and incubated again for 10 min at 60 °C. After incubation, 100 µl isopropanol was added to the tube and then the liquid transferred into the binding column, and centrifuged for 1 min at 8000 rpm. This step was repeated using 500 µl for both washing buffer 1 and 2; then, DNA was precipitated using 100 µl elution buffer and centrifugation at 13000 rpm for 1 min. Extracted DNA was kept at -20 °C until use in PCR. Conventional PCR reaction was done for detection of *Salmonella* spp., *Arcobacter* spp., and *Campylobacter* genus using specific primers (Table 1).

Table 1. The primers used in this study for detection of *Salmonella*, *Campylobacter* and *Arcobacter*

Primer sequence (5' to 3')	Target gene	Annealing temperature (°C)	Product size (bp)
F:ATCTAATGGCTTAACCATTAAAC R: GGACGGTAACTAGTTTAGTAT	16S rRNA (*Campylobacter* spp.)	59	875
F: GTGAAATTATCGCCGCCACGTTCGAA R: TCATCGCACCGTCAAAGGAACC	*Inv A* (*Salmonella* spp.)	58	284
F: AGAACGGGTTATAGCTTGCTAT R: GATAVAATACAGGCTAATCTCT	16S rRNA (*Arcobacter* spp.)	44	181

The PCR reaction mixtures consisted of 100 ng DNA template, 2.5 µl 10x PCR buffer (75 mM Tris HCl, pH 9.0, 2 mM MgCl2, 50 mM KCl, 20 mM (NH4)2SO4; Bioneer, Daejeon, So uth Korea), 0.2 mM dNTPs (Bioneer, Daejeon, South Korea), 1.5 U AmpliTaq DNA polymerase (Bioneer, Daejeon, South Korea), and 10 pmol each primer (Takapouzist, Tehran, Iran). The volume of the reaction mixture was reached to 25 µl using distilled deionized water. The thermal cycler (MJ mini, BioRad, USA) was adjusted under optimum conditions. Briefly, Initial denaturation at 94 °C for 4 min, followed by 33 cycles of denaturation at 94 °C for 1 min, annealing as shown in Table 1 for 1 min and extension at 72 °C for 1 min. Final extension was carried out at 72 °C for 7 min. Amplified products were separated by electrophoresis in 1.5% agarose gel electrophoresis stained with ethidium bromide (Cinnaclone, Tehran, Iran). The 100 bp DNA ladder was used as molecular size marker.

Statistical analysis: Using Chi-squared, all statistical analyses were performed by SPSS Inc., Chicago, IL (v. 18.0). P value less than 0.05 was considered for statistical significance.

RESULTS

Among 174 fecal samples collected from pet birds, a total of 114 (65.5%), 28 (16%), and 86 (49.4%) samples were found positive for *Campylobacter*, *Salmonella*, and *Arcobacter*, respectively. Also, simultaneous contamination with *Campylobacter* and *Salmonella* were shown in 12 (6%) of samples. 50 (28%) and 2 (1%) of samples were positive with dual infection of *Campylobacter* and *Arcobacter*, and *Salmonella* and *Arcobacter*, respectively. In 14 (8%) of samples, the presence of all three bacteria were confirmed. Results are summarized in Table 2 and Figures 1 and 2.

Table 2. Overall percentages of different types of bacteria isolated from fecal samples collected from pet birds

Birds (Scientific name)	Name of Bacteria		
	Campylobacter	Salmonella	Arcobacter
Linnet (*Carduelis cannabina*)	2/4 (50%)	0/4 (0%)	4/4 (100%)
Goldfinch (*Carduelis carduelis*)	12/16 (75%)	0/16 (0%)	12/16 (75%)
Rosy-faced lovebird (*Agapornis roseicollis*)	8/8 (100%)	0/8 (0%)	4/8 (50%)
Rose-ringed parakeet (*Psittacula krameri*)	4/6 (66.6%)	0/6 (0%)	4/6 (66.6%)
Canary (*Serinus canaria*)	4/6 (66.6%)	2/6 (33.3%)	6/6 (100%)
Cockatiel (*Nymphicus hollandicus*)	10/14 (71.4%)	2/14 (14.2%)	6/14 (42.8%)
Common mynah (*Acridotheres tristis*)	2/12 (16.6%)	2/12 (16.6%)	4/12 (42.8%)
Budgerigar (*Melopsittacus undulatus*)	8/20 (40%)	2/20 (10%)	8/20 (40%)
Grey parrot (*Psittacus erithacus*)	4/8 (50%)	2/8 (25%)	2/8 (25%)
Alexandrine parakeet (*Psittacula eupatria*)	2/2 (100%)	0/2 (0%)	0/2 (0%)
Blue-winged parrot (*Neophema chrysostoma*)	2/2 (100%)	0/2 (0%)	0/2 (0%)
Eastern rosella (*Platycercus eximius*)	2/2 (100%)	0/2 (0%)	0/2 (0%)
Sun conure (*Aratinga solstitialis*)	2/2 (100%)	0/2 (0%)	0/2 (0%)
Blue-and-yellow macaw (*Ara ararauna*)	2/2 (100%)	0/2 (0%)	0/2 (0%)
Diamond dove (*Geopelia cuneata*)	2/2 (100%)	0/2 (0%)	2/2 (100%)
Rock pigeon (*Columba livia*)	8/10 (80%)	4/10 (40%)	0/10 (0%)
Old dutch capuchine (*Columba livia*)	2/6 (33.3%)	0/6 (0%)	4/6 (66.6%)
Fantail pigeon (*Columba livia*)	26/34 (76.4%)	14/34 (41.1%)	18/34 (52.9%)
White-eared bulbul (*Pycnonotus leucotis*)	12/18 (66.6%)	0/18 (0%)	12/18 (66.6%)
Total	**114/174 (65.5%)**	**28/174 (16%)**	**86/174 (49.4%)**

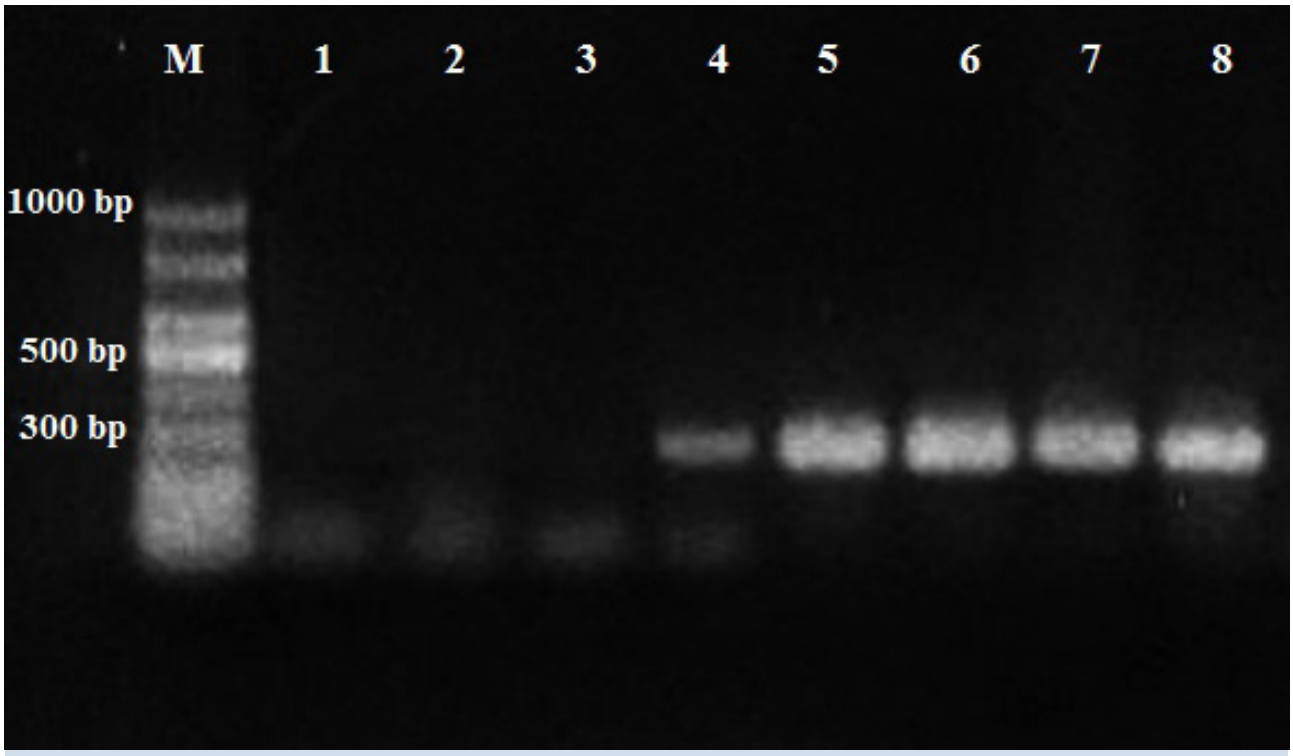

Fig. 1. Agarose gel electrophoresis of PCR of the *invA* gene (284 bp) for the characterization of the *Salmonella* species. M: 100-bp ladder as molecular DNA marker; Lane 3: negative control; Lane 4: positive control; Lanes 5-8: positive samples.

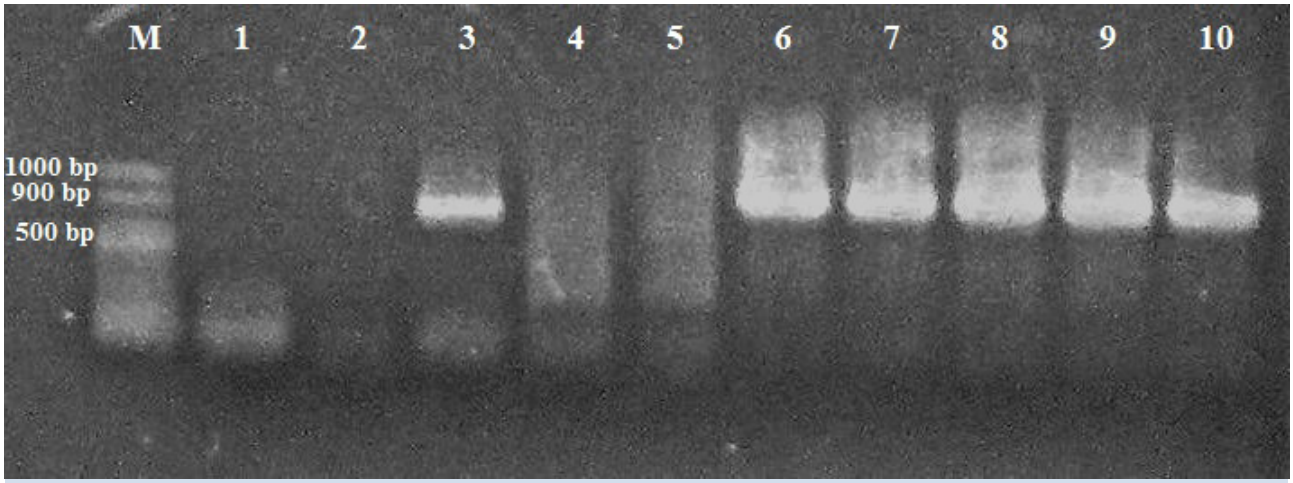

Fig. 2. Agarose gel electrophoresis of PCR amplification products using 16S rRNA (857 bp) as a specific primer to identify the *Campylobacter* species. M: 100-bp ladder as molecular DNA marker; Lane 2: negative control; Lane 3: positive control; Lanes 4-5: negative samples; Lanes 6-10: positive samples.

DISCUSSION

The risk of getting a disease from pet bird is typically highest in people who already have chronic diseases, such as the very young, the elderly, HIV-infected individuals, organ-transplant recipients, and people receiving chemotherapy. Some of the most commonly reported bacterial diseases that can be transmitted through pet birds include Salmonellosis, and Campylobacteriosis. Thus, diagnosis and control of mentioned disease in birds is very important.

Birds are usually considered to be the reservoir of *Campylobacter*, because the body temperature of the birds provides conditions for bacterial growth (Dhama et al., 2013). The overall prevalence of *Campylobacter* spp. for all pet birds sampled in this study was 65.5%. However, the prevalences of *Campylobacter* spp. in members of different pet bird families were

from 16.6% to 100%. Compared with the results of other studies describing *Campylobacter* in pet bird populations, which reported prevalences ranging from 0% to 50% (Waldenstrom et al., 2002; Colles et al., 2008; Zamani Moghaddam et al., 2011; Ehsannejad et al., 2015), the prevalence reported in this study is relatively high. Prevalence differences between studies may due to the use of different sampling and culture methods, which vary in sensitivity. High prevalence rate of *Campylobacter* Spp. (75%) in broiler flocks of Iran was reported by Ansari-Lari et al. (2011), but low detection rate of these bacteria from pet birds mentioned in a previous study (Ehsannejad et al., 2015). In a recent research, Dipineto et al. (2017) reported 13.6% of the cage samples of pet birds were positive for *Campylobacter coli*. One of the possible reasons for the difference in the prevalence can be due to the difference between the bird species. Survival of *Campylobacter* spp. in fecal samples from different bird species is variable (Waldenstrom et al., 2007). Moreover, the type of sample (e.g., cloacal swab vs. fecal sample) and time of sampling (e.g., seasonal variation) collected from birds to assess the prevalence of this organism can influence research findings. The sensitivity of culture techniques should also be considered as a source of variation for prevalence estimates, especially given the fastidious nature of organisms such as *Campylobacter* (Mi'kanatha et al., 2012).

Out of 174 sample tested, 28 samples (16%) were positive for *salmonella* spp. *Salmonella* were detected from Canary, Cockatiel, Common mynah, Budgerigar, Grey parrot, Rock pigeon and Fantail pigeon. Results of present study showed high prevalence of *Salmonella* spp. in pet birds in comparison of other researches. Rahmani et al. (2011), reported from 668 samples tested from birds kept in parks and pet shops in Tehran, Iran, 19 isolates (2.8%) were identified. Similar to some previous study, high prevalence of *Salmonella* spp., was shown in canaries (Georgiades and Iordanidis, 2002; Madadgar et al., 2009; Rahmani et al., 2011). On the other hand, our results is in line with results of Brobey et al. (2017), which reported 17% infection rates of *Salmonella* in wild birds from southeast Texas. In current research, high prevalence of *Salmonella* was detected in pigeons. These results are in agreement to Osman et al. (2013) who reported 33.3% incidence in pigeons. Lower incidence rates were recorded by other researchers who recorded incidence rates of 0%, 3.9%, 4% and 7.9%, respectively (Lillehaug et al., 2005; Tanaka et al., 2005; González-Acuña et al., 2007; Sousa et al., 2010).

High percentage of *Campylobacter* and *Salmonella* detection in this study may be due to geographical and environmental connection. Mazandaran is in the north of Iran (53°6′E, 36°23′N) and located along the southern coast of the Caspian Sea which shares borders with Russia, Kazakhstan, Azerbaijan, and Turkmenistan. Each year when the cold seasons arrive, migratory birds come to Mazandaran wetlands and stay until early March. It is possibility that migratory birds to be an important sources for mentioned bacteria and moving of migratory bird can help to spread infection by direct or indirect contact with carrier, reservoir, Salmonella-infected animals and birds.

Regarding to detection of *Arcobacter* in cloacal swabs, in our study, the highest detection was in Linnet, Canary, Diamond dove, and Goldfinch with incidence rates of 100% for the first three and 75% for the last. Data about the presence of *Arcobacter* spp. in wild birds are rare. This is the first report of *Arcobacter* detection in pet birds in Iran. Di Francesco et al. (2014) showed that 19% detection of *Arcobacter* from Eurasian collared doves in Northern Italy. Khoshbakht et al. (2017) reported no detection of *Arcobacter* from quail farms in Northern Iran. Some researchers showed high isolation of *Arcobacter* from chicken farms (Son et al., 2007; Ho et al., 2008).

In conclusion, this study shows that *Campylobacter*, *Salmonella*, and *Arcobacter* may be excreted in the faeces of apparently healthy pet birds; therefore, pet birds may be a potential source of these bacteria transmission to humans. Furthermore, some birds showed contamination with two or all three of these bacteria. To our knowledge, the molecular detection of *Arcobacter* was reported for the first time in pet birds in Iran. Close physical contact with pet birds that are uncertain about their status, can be a potential risk for public health.

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CONFLICT OF INTEREST

The authors do not have any potential conflicts of interest to declare.

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