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Molecular Identification of *Campylobacter*, *Arcobacter*, and *Salmonella* in Japanese Quail (*Coturnix japonica*) Reared in Farms of Northern Iran

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HIGHLIGHTS

- Prevalence rates of *Campylobacter*, *Salmonella*, and *Arcobacter* in faecal samples were 95, 65 and 0%, respectively.
- Prevalence of *Salmonella* in rural farms was significantly higher than semi industrial farms.
- Risk of *Campylobacter* and *Salmonella* among quail farmed in Northern Iran should be highlighted.

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Acronyms and abbreviations
PCR=Polymerase Chain Reaction

ABSTRACT

Background: Food animals such as different rearing birds can transmit zoonotic enteropathogenic bacteria, which exist in their intestinal microbiota. This research was designed in order to molecular identification of *Campylobacter*, *Arcobacter*, as well as *Salmonella* in Japanese quail (*Coturnix japonica*) reared in farms of Northern Iran.

Methods: Total of 100 cloacal samples were collected from 20 different quail farms. After extraction of total DNA, the samples subjected to molecular detection of the *Campylobacter*, *Arcobacter*, and *Salmonella* using polymerase chain reaction. By Chi-squared, all statistical analyses were performed by SPSS Inc., Chicago, IL (v. 18.0).

Results: Totally, the prevalence rates of *Campylobacter*, *Salmonella*, and *Arcobacter* in samples were 95, 65, and 0%, respectively. The prevalence of *Salmonella* spp. in rural farms was significantly (p<0.05) higher than semi-industrial farms.

Conclusion: High occurrence of *Campylobacter* spp. and *Salmonella* spp. were found in rearing quail populations of Mazandaran province, Northern Iran. These enteropathogens can contaminate food products obtained from the birds indicating their public health importance.

Introduction

In recent years, rearing of birds other than chickens has been developed all around the world to supply the food requirements of the human. One of these kinds of birds is quail particularly Japanese quail (*Coturnix japonica*), one of the subspecies of common quail (*Coturnix coturnix*), which is the usual rearing quail species in developing countries like Iran. It means that such birds not only are not considered as game birds today but also are the important source of protein in developing countries.

As we know, when animals farmed as human food, there are some essential aspects which should be considered and studied in point of view of the human health and safety. One of these features is the role of these animals in the spread of zoonotic microorganisms; particularly human enteric pathogens which are transmitted to human through food animals (Benskin et al., 2009). Different sources during rearing, slaughter, and processing can transmit the bacterial contamination into the carcass and meat of the birds. But it seems that the main source of the contamination is the viscera as well as intestine of the birds which contains various microorganisms, including

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human enteric pathogens such as *Escherichia coli*, *Salmonella*, as well as *Campylobacter* (Abdollahpour et al., 2015; Dipineto et al., 2014; Vashin and Stoyanchev, 2005). There is limited information about the presence of various human enteric pathogens in the intestinal flora of rearing quail and related farms in Iran. So, this research was designed in order to molecular identification of *Campylobacter*, *Arcobacter*, and *Salmonella* in Japanese quail (*Coturnix japonica*) reared in farms of Northern Iran.

Materials and methods

Sample collection

During June to July 2015, 100 fresh faecal samples were randomly collected from 20 (five samples for each farm) different rural (n=8), industrial (n=1), and semi-industrial (n=11) Japanese quail farms in Mazandaran province, Northern Iran. Sampling was done from all selected birds which were in slaughter stage with 35 to 40 days old. Faecal samples were obtained from cloaca region of the birds using sterile gloves and swabs, collected in trypticase soy broth (Merck KGaA, Darmstadt, Germany) tubes, and transported to microbiological laboratory, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Iran.

Positive controls

One g of each sample from a farm was pooled and homogenized using rotor-stator homogenizer by sterile plastic pestle to prepare for stool DNA extraction. The extracted DNA from strains of *Campylobacter jejuni* (ATCC 33291), *Arcobacter butzleri* (ATCC 49616), as well as *Salmonella enterica* serotype Typhimurium (local isolate) were considered as positive controls in the Polymerase Chain Reaction (PCR) test.

DNA extraction

DNA extraction from pooled samples 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.

PCR assay

Conventional PCR reaction was done for detection of *Salmonella* spp., *Arcobacter* spp., and *Campylobacter* genus using specific primers (Table 1). 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 $MgCl_2$, 50 mM KCl, 20 mM $(NH4)_2SO_4$; 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

Totally, the prevalence rates of *Campylobacter*, *Salmonella*, and *Arcobacter* in samples were 95, 65, and 0%, respectively. Prevalence of *Salmonella* spp. in rural farms was significantly ($p<0.05$) higher than semi-industrial farms (Table 2).

Discussion

With raising the requirement of human to protein sources and different meats, the role of the birds for resolving this problem has increased and today, some previously game birds such as quails and partridges are reared in industrial or non-industrial farms as food animals. All of these food animals and particularly birds, are significant vectors for different infectious diseases especially, enteropathogenic agents which some of them are intestinal flora of animals and so transmit to human through contamination of animal related food products, processing, and producing industries and their instru-

ments (Benskin et al., 2009; Khoshbakht et al., 2014; Rahimi et al., 2010). In present study, the high frequency of quail farms was positive for the presence of *Campylobacter* spp. in intestinal samples of the rearing birds, while *Arcobacter* spp. was not detected in cloacal samples. *Campylobacter* and *Arcobacter* are Gram-negative, motile, and non-spore forming bacteria belonging to the Campylobacteraceae family which can cause gastroenteritis and some other disorders in human. One of the most important differences between *Arcobacter* and *Campylobacter* genus is the optimum growth temperature which *Arcobacter* can grow at 15-25 °C whereas human pathogenic *Campylobacter* species are thermophiles (Humphrey et al., 2007; Iovine et al., 2008; Vandamme et al., 1991).This difference can be the main reason for the negative result in detection of *Arcobacter* spp. in Japanese quail samples found in the present work; because the body temperature of this bird is about 41-43 °C that is not suitable for growth of *Arcobacter* spp. (Gilbreath and Ko, 1970); while the *Campylobacters* especially, their thermophile species can truly grow in this condition.

Table 1: The primer pairs used in this study for detection of three human enteropathogenic bacteria, including *Campylobacter*, *Salmonella*, *Arcobacter*

Primer sequence (5' to 3')	Target gene	Annealing temperature (°C)	Product size (bp)	Reference
F: ATCTAATGGCTTAACCATTAAAC R: GGACGGTAACTAGTTTAGTAT	16S rRNA (*Campylobacter* spp.)	59	857	(Linton et al., 1997)
F: GTGAAATTATCGCCGCCACGTTCGAA R: TCATCGCACCGTCAAAGGAACC	*invA* (*Salmonella* spp.)	58	284	(Rahn et al., 1992)
F: AGAACGGGTTATAGCTTGCTAT R: GATACAATACAGGCTAATCTCT	16S rRNA (*Arcobacter* spp.)	44	181	(Gonzalez et al., 2000)

Table 2: Prevalence of the three common enteropathogenic bacteria, including *Campylobacter*, *Salmonella*, *Arcobacter* in quail reared in farms of Northern Iran

Number of farm	Name of pathogens			Type of farm
	Campylobacter spp.	*Salmonella* spp.	*Arcobacter* spp.	
1	-	-	-	Industrial
2	+	-	-	Semi-industrial
3	+	+	-	Semi-industrial
4	+	+	-	Rural
5	+	+	-	Rural
6	+	-	-	Semi-industrial
7	+	+	-	Rural
8	+	+	-	Rural
9	+	-	-	Semi-industrial
10	+	+	-	Semi-industrial
11	+	+	-	Rural
12	+	-	-	Semi-industrial
13	+	+	-	Rural
14	+	+	-	Semi-industrial
15	+	+	-	Semi-industrial
16	+	-	-	Semi-industrial
17	+	-	-	Semi-industrial
18	+	+	-	Rural
19	+	+	-	Rural
20	+	+	-	Semi-industrial
Total	19	13	0	-

Some other studies previously had reported the prevalence of *Campylobacter* spp. in quail feces or cloacal samples in the range of 21 to 80% (Dipineto et al., 2014; Mirzaie et al., 2011; Vashin and Stoyanchev, 2005; Vashin et al., 2008), whereas the prevalence of the microorganism was reported significantly lower in quail related food products such as egg or meat (McCrea et al., 2006; Vashin and Stoyanchev, 2005). It means that the main source of the contamination is intestinal microbiota; also, contamination is often decreased during processing and production of the foods. Although we could not detect *Arcobacter* spp. in cloacal samples recovered from the Japanese quail, other studies formerly have reported the presence of this microorganism in quail organs and meat in Iran (Rahimi, 2014).

Further researches are necessary for conclusive consideration about the presence of the *Arcobacter* spp. in the Japanese quail. Consumption of contaminated poultry meat and egg is the most important cause of *Salmonella* infections in human (Carli et al., 2001). *Salmonella* spp. have been frequently detected in food bird products and different reports have been published regarding the prevalence of *Salmonella* serotypes (Harsha et al., 2011; Rahimi et al., 2010), but results of the present study showed considerably higher prevalence of *Salmonella* among quail farms in comparison with other similar studies (de FreitasNeto et al., 2013; Palanisamy and Bamaiyi, 2015). Furthermore, in the current study, the distribution of *Campylobacter* and *Salmonella* in combination among rural farms of Japanese quail was significantly higher than semi-industrial farms. In rural farms in North of Iran, farmers often breed different birds in one field or in close areas, particularly in combination with geese and ducks which are the common and main sources of some enteropathogenic bacteria (Jamali et al., 2014; Moriarty et al., 2011).

Conclusion

High occurrence of *Campylobacter* spp. and *Salmonella* spp. were found in rearing quail populations of Mazandaran province, Northern Iran. These infectious bacteria can contaminate food products obtained from the birds indicating their public health importance. So, it is essential to evaluate different aspects of this potential risk such as antibiotic resistance level of microorganisms or determining the presence of specific pathogenic strains.

Conflicts of interest

The authors have no conflict of interest.

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