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Isolation and Molecular Identification of *Avibacterium paragallinarum* Isolated from Commercial Layer and Backyard Chickens in Iran

Seyed Mostafa Peighambari[1*], Abbas Nouri[2], Reza Barzegari[1], Amir Ghorbani[3]

1. *Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
2. *Department of Avian Disease Research and Diagnostic, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*
3. *Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University, Columbus, Ohio, USA*

Abstract

BACKGROUND: *Avibacterium paragallinarum* causes infectious coryza that is an important disease of chickens associated with an acute upper respiratory infection, growth retardation, marked drop in egg production, reduced hatchability, and increased number of culls. Infectious coryza has been reported from all around the world including Iran where chickens are raised.

OBJECTIVES: This study was conducted to isolate *Avibacterium paragallinarum* from the suspected cases of infectious coryza in the backyard and commercial layer chickens in three provinces of Tehran, Alborz, and Qazvin in Iran, to characterize the isolates by molecular methods and to determine their antimicrobial susceptibility profile.

METHODS: Swab samples from eye secretions and mouth cavity were provided from five commercial laying farms (25 samples) and backyard chickens (20 samples) suspected of infectious coryza in Tehran, Alborz, and Qazvin provinces of Iran. Standard bacteriological and biochemical procedures were performed for the isolation and identification of *Av. paragallinarum*. The antimicrobial susceptibility of the recovered isolates was determined by the agar disk diffusion method. HPG-2 PCR was used to confirm *Av. paragallinarum* using the specific primers of N1 and R1, resulting in a 500 bp amplicon. PCR-amplified *hmtp210* gene with the amplicon size of 1.6 kb was subjected to sequencing with the standard Sanger sequencing method and phylogenetically analyzed.

RESULTS: Three *Av. paragallinarum* isolates were obtained from all the cultured samples. Antimicrobial sensitivity test revealed that all three isolates were resistant to amoxicillin, oxytetracycline, streptomycin, ampicillin, and colistin while they were susceptible to cephalexin, ceftriaxone, florfenicol, gentamicin, linco-spectin, neomycin, and doxycycline. In phylogenetic analysis, two different genotypes of *Av. paragallinarum* were found.

CONCLUSIONS: In this study, *Av. paragallinarum* isolates recovered from layer flocks were genotypically closely related to the strains from serovar C while the isolate recovered from backyard chickens was closely related to serovar B strains. Our results showed that the antimicrobial susceptibility is highly variable among the *Av. paragallinarum* isolates compared to previous studies. Future studies are required to develop better immunogenic strategies and eventually novel vaccine candidates to control the disease and reduce the risk of infection with multi-drug resistant *Av. paragallinarum* isolates.

KEYWORDS: *Avibacterium paragallinarum*, *hmtp210* gene, Infectious coryza, Poultry, Phylogenetic analysis

Correspondence

Seyed Mostafa Peighambari, Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Tel: +98 (21) 61117150, Fax: +98 (21) 66933222, Email: mpeigham@ut.ac.ir

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Introduction

Avibacterium paragallinarum is a Gram- negative bacterium and the causative agent of infectious coryza, an important disease of chickens associated with an acute upper respiratory infection, growth retardation, marked drop in egg production (10-40%), reduced hatchability, and increased number of culls. Infectious coryza is normally characterized by the acute onset, very rapid spread in the flock, and by the signs of nasal discharge, facial swelling, lacrimation, anorexia, and diarrhea (Blackall and Soriano-Vargas, 2020). Horizontal transmission within the flocks occurs through aerosols and direct contact. However, vertical transmission from parents to progeny stocks has yet to be documented. Therefore, the intermittent excretion of *Av. paragallinarum* from the carrier birds with subclinical infections plays an important role in spreading the disease (Blackall and Soriano-Vargas, 2020). Infectious coryza has been reported from all around the world including Iran where chickens are raised (Badouei *et al.*, 2014; Nouri *et al.*, 2014; Patil *et al.*, 2017; Crispo *et al.*, 2018; Wahyuni *et al.*, 2018; Nouri *et al.*, 2021).

Antimicrobial agents may be used to treat the diseased flocks in order to reduce the severity and the spread of the disease. However, generally, prevention of infectious coryza mainly relies on good biosecurity practices and vaccination in poultry flocks. Despite these measures, sporadic infectious coryza outbreaks continue to occur and pose significant economic losses to the poultry industry especially in developing countries. Inactivated whole-cell vaccines against infectious coryza are widely used however their protection is only limited to the serovars used to prepare the vaccine (Blackall and Soriano-Vargas, 2020). In Iran, despite extensive vaccination programs against infectious coryza, the disease is frequently observed in the poultry farms. Therefore, unvaccinated poultry flocks and the farms that do not strictly follow the biosecurity principles and/or have not been properly vaccinated, are at risk. Moreover, broilers that are not usually vaccinated are at risk of exposure and infection (Christensen *et al.*, 2002).

Despite the widespread occurrence of the disease in Iran, the genotypic characteristics of *Av. paragallinarum* in isolates circulating among poultry farms are largely unknown. Therefore, the aims of this study were molecular characterization and determination of antimicrobial susceptibility patterns of *Av. paragallinarum* isolated from the suspected cases of infectious coryza in backyard and commercial layer chickens in three provinces of Tehran, Alborz, and Qazvin in Iran.

Materials and Methods

Sample Collection

Swab samples were provided from five commercial laying farms (25 samples) and backyard chickens (20 samples) in which suspected chickens to infectious coryza were found during autumn and winter between November 2018 and May 2019. Swabs were taken from eye secretions and mouth cavity of suspected birds and placed in a tube containing pre-prepared NAD-enriched brain-heart infusion (BHI) medium and transferred to the laboratory in less than 24 hours. Only farms with obvious symptoms including unilateral or bilateral facial swelling, subcutaneous sinus swelling, tearing, and rhinorrhea were sampled. Sampled farms were located in Tehran, Alborz, and Qazvin provinces of Iran.

Bacteriological Culture

Each swab sample was streaked onto a sheep blood agar plate, then one or two intersecting lines of *Staphylococcus aureus* as a nurse bacterium was made on the medium. The inoculated plates were incubated at 37°C and 5% CO_2 for 24 hours. The small colonies with satellite growth as a single colony were carefully searched and pure colonies were isolated by re-culturing onto the sheep blood agar plates as described above. The purified colonies were cultured onto 7% Colombian horse blood agar (QUELAB, Canada). Biochemical reactions were performed to determine catalase and oxidase activities. Three *Av. paragallinarum* confirmed isolates were lyophilized and frozen in glycerol at -80°C until further use.

Antimicrobial Susceptibility Test

The antimicrobial susceptibility of the *Av. paragallinarum* isolates to a panel of antimicrobial agents was assessed through the agar disk diffusion method

on 7% Colombian horse blood agar. The following antimicrobials (concentrations in µg) were tested: cephalexin (30), ceftriaxone (30), ampicillin (10), amoxicillin (30), neomycin (30), streptomycin (10), gentamicin (10), florfenicol (30), oxytetracycline (30), doxycycline (30), linco-spectin (15/200), colistin (10), and trimethoprim-sulfamethoxazole (1.25/23.75). The *Escherichia coli* ATCC 25922 reference strain was included for quality control. All antibacterial disks were provided from Padtan Teb Co. (Tehran, Iran). Briefly, a bacterial suspension from an overnight culture was adjusted to a turbidity of 0.5 McFarland standards and spread onto 7% Colombian horse blood agar plates. The antimicrobial disks were then placed on the plates and incubated at 37°C and 5% CO_2 for 24 hours. The interpretation of results was carried out according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018).

Identification of *Avibacterium paragallinarum* by PCR

The colonies suspected to be *Av. paragallinarum* were subjected to HPG-2 PCR using primers N1 and R1 resulting to a 500 bp amplified product as shown in Table 1 (Chen *et al*., 1996; Nouri *et al*., 2021). No known function has been attributed to HPG-2 amplicon sequence. The extraction of bacterial DNA was performed by phenol-chloroform method as described by Moore & Dowhan (2002). By the use of a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher, USA), the extracted DNA samples were measured in terms of quantity and purity. The specific primers were synthesized by SinaClon (Tehran, Iran). Amplification reactions were carried out in a 25 µL reaction volume containing 12.5 µL of Mastermix, 0.5 µL of each of the forward and reverse primers, and 6.5 µL of deionized water. Approximately 4 µL of template DNA was added to the mixture. DNA extracts from the cultures of *Av. paragallinarum* and *Ornitobacterium rhinotracheale* (ORT) were included in all PCR reaction sets, respectively, as positive and negative controls. Amplification was performed in a thermocycler (Mastercycler, Eppendorf, Germany) as follows: 95°C for 7 min followed by 35 cycles of 94°C for one min, 58°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 7 min. The amplification products were detected by 1% agarose gel electrophoresis in 1 x TAE buffer.

Sequence and Phylogenetic Analysis

The *hmtp210* gene of *Av. paragallinarum* encodes an outer-membrane hemagglutinin (HA) that has an important role in the pathogenicity of this bacterium (Araya-Hidalgo *et al.*, 2017). In all three *Av. paragallinarum* isolates, the *hmtp210* gene was amplified using specific primers (Table 1) and protocol as described previously (Sakamoto *et al.*, 2012). Amplified PCR products were subjected to sequencing with the standard Sanger sequencing method at the Macogen Company (Seoul, South Korea). Blast analysis was performed against the available sequences from *Av. paragallinarum* strains on the NCBI GenBank database (n=100, accessed on August 2020). Phylogenetic analysis of the *hmtp210* genes was done using the Neighbor-Joining and Maximum Composite Likelihood method with 1000 bootstrap replicates following ClustalW alignment option in MEGA-X, version 10.0.5 (Kumar *et al.*, 2018). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Table 1. Primer sets used for amplification of the DNA fragments in this study

Primer set	Primer	Primer sequence	Product size
HPG-2	R1 - Forward	5'-CAAGGTATCGATCGTCTCTCTACT-3'	500 bp
	N1 - Reverse	5'-TGAGGGTAGTCTTGCACGCGAAT-3'	
***hmtp210* gene**	5-1 - Forward	5'-GATGGCACAATTACATTTACA-3'	1.6 kbp
	5-1 - Reverse	5'-ACCTTGAGTGCTAGATGCTGTAGGTGC-3'	

Results

Bacteriological Findings and Antimicrobial Sensitivity Profiles

In total, three *Av. paragallinarum* isolates were obtained from all the cultured samples. *Av. paragallinarum* isolated from the commercial and backyard flocks in Iran demonstrated similar antimicrobial sensitivity profiles. All three isolates were resistant to amoxicillin, oxytetracycline, streptomycin, ampicillin, and colistin. Additionally, all the isolates were susceptible to cephalexin, ceftriaxone, florfenicol, gentamicin, lincospectin, neomycin, and doxycycline. Furthermore, all three isolates showed intermediate susceptibility to trimethoprim sulfamethoxazole.

Sequence and Phylogenetic Analysis

Two different genotypes of *Av. paragallinarum* are responsible for infections in the commercial and backyard flocks in Iran. As shown in Figure 1, the results of phylogenetic analysis on the *hmtp210* genes showed that the *Av. paragallinarum* strains isolated from backyard (IR-98-5nat; MN928968) and commercial layer (IR-98-1alay and IR-98-1blay; MN928969 and MN928970, respectively) flocks in this study were closely related to two different reference strains from Germany (strain 2671; KU143740; serovar B-1) and Australia (strain HP60; KU143744; serovar C-4), respectively. Accordingly, the isolates from backyard (IR-98-5nat) and commercial layer (IR-98-1alay and IR-98-1blay) flocks shared between 100% and 99.82% *hmtp210* genes nucleotide sequence identity with the German strain 2671 and Australian strain HP60, respectively (Figure 2). Interestingly, both of the isolates from the commercial layer flocks (IR-98-1alay and IR-98-1blay) possessed an insertion of 138 nucleotides which corresponded to nucleotides from 3504 to 3641 from the *hmtp210* genes of the Australian strain HP60 (Figure 3). A similar insertion was previously reported for other Australian (HP31; KU167070) and Taiwanese strains (TW07, TW08, and TW13; KJ867498, MT050502 and MT050506, respectively) (Wang *et al.*, 2016; Tan *et al.*, 2020). Collectively, these results emphasized that at least two different genotypes of *Av. paragallinarum* circulated among Iranian poultry flocks.

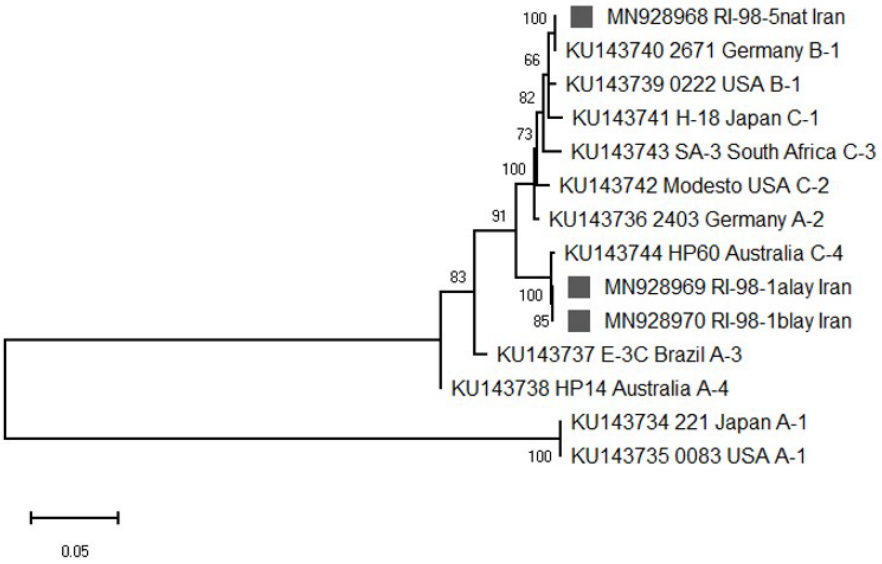

Figure 1. Neighbor-Joining tree compared to reference strains for all serovars, MEGA-X v10.0.5. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. This analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1882 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MN928968_RI-98-5nat_Iran 1		87.67	87.67	47.35	47.28	98.69	92.88	91.38	98.63	100.00	98.24	98.30	97.65	87.61
MN928969_RI-98-1alay_Iran 2	0.13		100.00	44.71	44.65	88.75	83.03	86.87	87.25	87.67	86.65	88.15	86.59	99.82
MN928970_RI-98-1blay_Iran 3	0.13	0.00		44.71	44.65	88.75	83.03	86.87	87.25	87.67	86.65	88.15	86.59	99.82
KU143734_221_Japan_A-1 4	0.91	1.00	1.00		99.93	47.47	46.95	47.14	47.49	47.35	47.68	47.47	47.62	44.65
KU143735_0083_USA_A-1 5	0.91	1.00	1.00	0.00		47.41	46.89	47.08	47.43	47.28	47.62	47.41	47.55	44.59
KU143736_2403_Germany_A-2 6	0.01	0.12	0.12	0.90	0.91		92.16	92.36	98.24	98.69	97.58	99.08	97.52	88.69
KU143737_E-3C_Brazil_A-3 7	0.07	0.19	0.19	0.92	0.92	0.08		95.71	92.10	92.88	91.44	91.90	91.18	82.97
KU143738_HP14_Australia_A-4 8	0.09	0.14	0.14	0.91	0.92	0.08	0.04		90.73	91.38	90.20	91.77	90.14	86.81
KU143739_0222_USA_B-1 9	0.01	0.14	0.14	0.90	0.91	0.02	0.08	0.10		98.63	98.82	97.71	98.22	87.19
KU143740_2671_Germany_B-1 10	0.00	0.13	0.13	0.91	0.91	0.01	0.07	0.09	0.01		98.24	98.30	97.65	87.61
KU143741_H-18_Japan_C-1 11	0.02	0.15	0.15	0.90	0.90	0.02	0.09	0.10	0.01	0.02		97.06	98.22	86.59
KU143742_Modesto_USA_C-2 12	0.02	0.13	0.13	0.90	0.91	0.01	0.09	0.09	0.02	0.02	0.03		97.65	88.09
KU143743_SA-3_South_Africa_C-3 13	0.02	0.15	0.15	0.90	0.90	0.03	0.09	0.11	0.02	0.02	0.02	0.02		86.54
KU143744_HP60_Australia_C-4 14	0.14	0.00	0.00	1.00	1.01	0.12	0.19	0.15	0.14	0.14	0.15	0.13	0.15	

Figure 2. Pairwise comparison with reference strains for all serovars, CLC Workbench v5.5

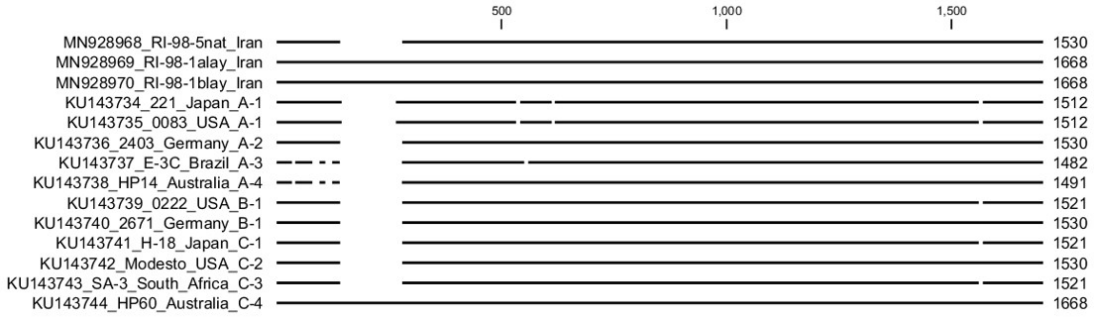

Figure 3. Sequence alignment compared to reference strains for all serovars, an overview of insertions/deletions, CLC Workbench v5.5

Discussion

Infectious coryza is an acute respiratory disease of chickens caused by *Avibacterium paragallinarum.* This disease is very contagious leading to acute symptoms in the upper respiratory tract of birds and becomes a chronic respiratory disease when accompanied by other pathogens (Blackall and Soriano-Vargas, 2020).

In recent years, due to the increased spread of chronic respiratory disease (CRD), it has become very important to investigate the pathogens in the respiratory system of birds. Unfortunately, due to the misdiagnosis in recognition of the causes of respiratory diseases, infectious coryza is not usually treated properly leading to huge waste of costs for the wrong treatment and possible increased antimicrobial resistance. Moreover, when infectious coryza occurs in poultry flocks of developing countries, owing to the presence of other pathogens and stress factors, more economic losses occur (Blackall and Soriano-Vargas, 2020).

In this study, three *Av. paragallinarum* strains were isolated from the poultry sources using the bacteriological culture and then confirmed by PCR using specific primers. Antimicrobial susceptibility patterns of three isolates were determined. The PCR-amplified *hmtp210* genes of three isolates were subjected to sequencing and more information on *Av. paragallinarum* isolates was revealed from Iran.

Due to the limited number of studies on *Av. paragallinarum* in Iran, investigation on the presence of *Av. paragallinarum* in the laying flocks of the country was the main objective of the current study, in which the presence of *Av. paragallinarum* in backyard and commercial laying flocks was verified. In another study conducted by Badouei *et al.*, (2014), 14 samples of laying hens were cultured. Out of five samples with suspected colonies to *Av. paragallinarum*, only one sample was verified as *Av. paragallinarum* using the PCR. Badouei *et al.* (2014) also did the PCR test on the swab sample of sinus and detected *Av. paragallinarum* in one backyard farm and two commercial laying flocks. These observations indicate the high sensitivity of PCR in the detection of positive clinical samples. Therefore, according to the results of the direct molecular tests and its comparison to the results of bacterial isolation, it is determined that PCR test on clinical

samples could be a reliable alternative for disease detection in poultry. The isolation and identification of *Av. paragallinarum* from backyard chickens which may be a source of infectious coryza for commercial chicken flocks have also been reported recently in Iran (Nouri *et al.*, 2021). Calderón *et al.* (2010) in Panama also reported that this disease could reduce egg production up to 45%, as well as an increase in losses in broiler breeder flocks. Muhammad and Sreedevi (2015), using the PCR techbique, detected *Av. paragallinarum* from outbreaks of infectious coryza in Andhra Pradesh of India (Muhammad and Sreedevi, 2015). From a total of 78 infraorbital sinus and nasal swabs, 56 samples (71.7%) were positive for infectious coryza. They found that the samples collected from chickens at acute stage of the disease or collected before treatment with antibacterial agents demonstrated better results on PCR. In Bangladesh, Khatun *et al.* (2016) worked on detection of *Av. paragallinarum* in 10 clinically sick broiler chickens during field outbreaks using the bacteriological culture of nasal and ocular discharges, tracheal swab, tracheal wash, and infraorbital sinus exudates. Based on colonial morphology, Gram staining reaction, sugar fermentation, and biochemical tests, only one isolate was confirmed as *Av. paragallinarum* among the samples collected from 10 broiler chickens. In the present study, no mortality was observed, indicating the efficacy of vaccines used in the sampled flocks.

Av. paragallinarum serotypes are typically determined by two related schemes (Blackall and Soriano-Vargas, 2020). The Page scheme is based on a slide agglutination test that differentiates the isolates into 3 serovars (A, B, and C). While the Kume scheme uses an HI test to classify the isolates into 9 serovars (A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3, and C-4) (Blackall and Soriano-Vargas, 2020). PCR has been extensively used for the molecular detection and identification of *Av. paragallinarum* (Chen *et al*., 1996; Miflin *et al.*, 1999). However, the species-specific PCR is not able to identify serogroups and/or serovars of *Av. paragallinarum.* Morales-Erasto *et al.* (2014) evaluated the ability of a multiplex PCR (mPCR) to differentiate the serogroups (A, B, and C) of *Av. paragallinarum*; however, due to the uncertainty about the accuracy of mPCR for recognition of the serogroups, the mPCR assay was not recommended to replace the conventional serotyping. In another study, Wang *et al.* (2016) examined mPCR as well as PCR followed by restriction fragment length polymorphism (RFLP) analysis as alternative approaches to replace the conventional serotyping by the Page scheme, but concluded that neither of the assays were appropriate for serotyping the *Av. paragallinarum* isolates. Between 2012 and 2013 in Korea, Han *et al.* (2016) isolated *Av. paragallinarum* from seven chicken farms and confirmed the isolates by PCR. They identified the isolates as serotype A using the mPCR.

In Iran, few studies have reported the prevalence of different *Av. paragallinarum* serotypes. Nouri *et al.* (2014), for the first-time, detected serotype B of *Av. paragallinarum* using the PCR with group -specific and Page serovar-specific primers that target the hypervariable region of haemagglutinin protein of *Av. paragallinarum.*

Currently, antimicrobial resistance is a worldwide public health concern (Nhung *et al.*, 2017). Increasing the knowledge on the antimicrobial resistance profile of *Av. paragallinarum* can prevent the improper use of antimicrobials in poultry flocks since this may result in the selection of resistant strains. There is not much published data on antimicrobial resistance of *Av. paragallinarum* in the scientific literature. In the present study, we evaluated the antimicrobial susceptibility of three *Av. paragallinarum* isolates to a panel of antimicrobial agents and found that the antimicrobial susceptibility profile was identical in all three isolates. In other regions, Han *et al.* (2016) evaluated the antibiotic sensitivity among *Av. paragallinarum* strains from seven chicken farms and demonstrated that only a few isolates appeared to be susceptible to erythromycin, gentamicin, lincomycin, neomycin, oxytetracycline, spectinomycin, and tylosin. In Bangladesh, Khatun *et al.* (2016) evaluated the antibacterial susceptibility of one isolated *Av. paragallinarum* against five agents including ciprofloxacin, azithromycin, gentamicin, ampicillin, and cefalexin and found that the isolate was sensitive to ciprofloxacin, azithromycin, and gentamicin and resistant to ampicillin and cefalexin. Antimicrobial susceptibility was variable among *Av. paragallinarum* isolates of above studies and there were similarities and differences between antimicrobial susceptibility profile of this study and

that of previous works (Han *et al.*, 2016; Khatun *et al.*, 2016; Jeong *et al.*, 2017; Nhung *et al.*, 2017; Heuvelink *et al.*, 2018).

Haemagglutinin is a 210-kDa protein (HMTp210) of *Av. paragallinarum* that is known to have an important role in the bacterial virulence and the protective immunity against the bacterium (Wang *et al.*, 2014). Purified and recombinant HMTp210 antigens are shown to protect the chickens against experimental challenge with *Av. paragallinarum* (Sakamoto *et al.*, 2013). In addition, a mutant strain lacking the HMTp210 antigen was less virulent and exhibited no haemagglutinin activity and failed to provoke haemagglutinin inhibition antibodies in infected chickens. This mutant showed reduced ability in some other biological functions as evaluated by Wang *et al.* (2014). The presence of a hypervariable region in the HMTp210 proteins in some isolates of Page serovars A and C was reported (Wu *et al.*, 2011). Hypervariable regions of *hmtp210* gene of serovars A and C strains are known to be the most antigenic region of the HMTp210 protein. This highly antigenic hypervariable region within the *hmtp210* gene has been proposed as a candidate for recombinant vaccine production (Wu *et al.*, 2011). However, there is a paucity of information about the genetic diversity, variability, and complexity of the *hmtp210* hypervariable region. Recently, Araya-Hidalgo *et al.* (2017) analyzed the *hmtp210* hypervariable region in 16 clinical isolates from Costa Rica by sequencing and compared those 16 clinical isolates with four vaccine strains and other *hmtp210* sequences available in GenBank. Except for one isolate, all other isolates demonstrated high similarity with 2 of 4 reference vaccine strains (Araya-Hidalgo *et al.*, 2017). In a recent study, Xu *et al.* (2019) characterized 28 *Av. paragallinarum* field isolates by the sequence analysis of the hemagglutinin gene and found that most field strains (25/28) were placed in the same cluster in the phylogenetic tree. Molecular characterization of the three isolates of this study was performed through sequencing of the *hmtp210* genes. Analysis of the *hmtp210* genes revealed two different lineages of the *Av. paragallinarum* in commercial and backyard flocks closely related to the bacterial isolates from serovars B-1 and C-4, respectively. The isolation of two different lineages of *Av. paragallinarum* from the backyard and commercial layer flocks suggested a limited role for backyard flocks in spreading the disease to commercial poultry flocks in Iran. Due to a shortage of sequence data on *A. paragallinarum* strains from various parts of the world, the above studies may serve as preliminary investigations for understanding the genetic variability and diversity of the hypervariable region of *hmtp210*.

Conclusion

In this study, *Av. paragallinarum* isolates recovered from layer flocks were genotypically closely related to serovar C strains while the isolate recovered from backyard chickens was closely related to serovar B strains. Our findings showed that despite vaccine administration to Iranian poultry flocks, infectious coryza may still occur in poultry flocks. The impact of poultry production types, including their proximity to backyard flocks, on the incidence of infectious coryza, needs more investigation. The prevalence of different serotypes in various parts of the country should be studied. Due to the possible antimicrobial susceptibility variability among the *Av. paragallinarum* isolates, antimicrobial susceptibility should be evaluated whenever these bacteria are isolated. Future studies on a larger pool of *Av. paragallinarum* isolates from different countries are required to obtain complete genetic data on the hypervariable region of *hmtp210* of *A. paragallinarum.* This additional information is necessary to develop novel diagnostics, vaccines, and therapeutics to better control the disease and reduce the risk of infection with multi-drug resistant *Av. paragallinarum* isolates in poultry flocks.

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Conflict of Interest

The authors declare they have no conflict of interest associated with this work.

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جداسازی و شناسائی مولکولی *آوی‌باکتریوم پاراگالیناروم* جدا شده از مرغان بومی و تخم‌گذار تجاری در ایران

سید مصطفی پیغمبری[۱*]، عباس نوری[۲]، رضا برزگری[۱]، امیر قربانی[۳]

[۱] گروه بیماری‌های طیور، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

[۲] گروه تحقیق و تشخیص بیماری‌های طیور، موسسه تحقیقات واکسن و سرم‌سازی رازی، سازمان تحقیق، آموزش و ترویج کشاورزی، کرج، ایران

[۳] گروه طب پیشگیری دامپزشکی، دانشکده دامپزشکی، دانشگاه ایالتی اهایو، کلمبوس، اهایو، ایالات متحده آمریکا

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زمینه مطالعه: *آوی‌باکتریوم پاراگالیناروم* باعث ایجاد کوریزای عفونی می‌شود که بیماری مهمی در طیور است که با عفونت حاد تنفسی فوقانی، کاهش رشد، افزایش تعداد وازده‌ها، کاهش قابل توجه تولید تخم مرغ و هچ همراه است. کوریزای عفونی در سراسر نقاط جهان که دارای صنعت پرورش طیور است از جمله ایران گزارش شده است.

هدف: این مطالعه برای جداسازی *آوی‌باکتریوم پاراگالیناروم* از موارد کوریزای عفونی در مرغان بومی و تخم‌گذار تجاری در استان‌های تهران، البرز و قزوین، تعیین خصوصیات جدایه‌ها با روش‌های مولکولی و تعیین حساسیت ضدمیکروبی جدایه‌ها انجام شد.

روش کار: نمونه‌های سواب از ترشحات چشم و حفره دهان از پنج مزرعه تخم‌گذار تجاری (۲۵ نمونه) و مرغان بومی (۲۰ نمونه) مشکوک به کوریزای عفونی در استان‌های تهران، البرز و قزوین تهیه شد. این نمونه‌ها با روش‌های استاندارد باکتری‌شناسی و بیوشیمیایی برای جداسازی و شناسایی *آوی‌باکتریوم پاراگالیناروم* انجام گرفت. حساسیت ضدمیکروبی جدایه‌ها با روش آگار دیسک دیفیوژن تعیین شد. برای تائید *آوی‌باکتریوم پاراگالیناروم*، از HPG-2 PCR از پرایمرهای اختصاصی N1 و R1 استفاده شد که منجر به تولید فراورده‌ای با ۵۰۰ جفت باز می‌شود. ژن *hmtp210* جدایه‌ها با استفاده از پرایمرهای اختصاصی با روش PCR افزوده‌سازی شدند و فراورده ای با اندازه ۱/۶ کیلوباز تولید نمودند که با روش توالی‌یابی استاندارد سنگر تعیین توالی و تجزیه و تحلیل فیلوژنتیکی شدند.

نتایج: در مجموع، سه نمونه *آوی‌باکتریوم پاراگالیناروم* از تمامی نمونه‌های کشت‌شده به دست آمد. حساسیت ضدمیکروبی نشان داد که هر سه نمونه به آموکسی‌سیلین، اکسی‌تتراسایکلین، استرپتومایسین، آمپی‌سیلین و کلیستین مقاوم و همگی به سفالکسین، سفتریاکسون، فلورفنیکل، جنتامایسین، لینکواسپکتین، نئومایسین و داکسی سیکلین حساس بودند. در آنالیز فیلوژنتیک، دو ژنوتیپ مختلف *آوی‌باکتریوم پاراگالیناروم* مشخص شد.

نتیجه‌گیری نهایی: یافته‌های این مطالعه نشان داد که از نظر ژنوتیپی جدایه‌های *آوی‌باکتریوم پاراگالیناروم* به‌دست‌آمده از مرغان تخم‌گذار تجاری به سویه‌های سروار C بسیار نزدیک بودند در حالیکه جدایه به‌دست‌آمده از مرغان بومی به سویه‌های سروار B ارتباط نزدیک داشتند. حساسیت ضدمیکروبی در مقایسه با مطالعات مشابه بسیار متنوع بود. مطالعات آینده برای توسعهٔ استراتژی‌های ایمنی‌زایی بهتر و در نهایت واکسن‌های جدید برای کنترل بیماری و کاهش خطر عفونت به جدایه‌های با مقاومت چندگانه *آوی‌باکتریوم پاراگالیناروم* مورد نیاز است.

واژه‌های کلیدی: *آوی‌باکتریوم پاراگالیناروم*، آنالیز فیلوژنتیک، ژن *hmtp210*، کوریزای عفونی، طیور

نویسندهٔ مسئول: سید مصطفی پیغمبری، گروه بیماری‌های طیور، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران ایمیل: mpeigham@ut.ac.ir