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Research Article

Contamination of quail flocks with *Ornithobacterium rhinotracheale* in West Azarbaijan province

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ABSTRACT

Background: *Ornithobacterium rhinotracheale* (ORT) is a bacterial respiratory diseases in birds, which along with other pathogenic factors, plays a crucial role in reducing the performance of breeding flocks and causes significant economic losses to the poultry industry. This study aimed to isolate the bacteria by microbial culture and molecular detection of ORT by polymerase chain reaction (PCR). 130 tracheal swabs were collected from 13 quail flocks that were kept in cages. After the extraction of DNA from swab materials, the band of 784 bp based on the 16S rRNA gene was investigated using specific primers. The bacteria were isolated from tracheal swabs of 3 flocks (23.07%) by culture method. Following DNA extraction from trachea samples, the presence of bacteria was confirmed in 4 of 13 flocks (30.76%) by PCR assay. The results of this study, for the first time, demonstrated the presence of infection in quail flocks in the West Azarbaijan province of Iran. The PCR was a suitable method for the diagnosing of ORT. Due to the proof of the presence of ORT infection, vaccination is necessary for quail breeding farms in the West Azarbaijan province of Iran.

1. Introduction

Ornithobacterium rhinotracheale (ORT) is an infectious disease in birds associated with respiratory disorders, death and, reduced flock performance. The severity of clinical symptoms and mortality during the course of disease is highly variable and can be affected by environmental-managerial factors, concurrent diseases and, secondary infections. ORT is a gram-negative, non-motile, highly pleomorphic, slow-growing, rod-shaped and, non-spore-forming bacterium that belongs to the 16S rRNA superfamily V. The infection has been reported worldwide from several species of birds, including poultry, ducks, geese, gulls, seagulls, ostriches, partridges, pheasants, pigeons, quails, black crows, and turkeys. The initial symptoms of the disease include lethargy, reduced consumption of water and food, weight loss, nasal secretion, cough, sneezing and sinusitis. In most cases, acute respiratory complications, grounding and, death can be observed. In young birds, due to infection in the brain and skull, the disease may cause sudden death without respiratory symptoms. In older birds, acute

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pneumonia is observed with a high percentage of mortality.

Due to osteomyelitis, lameness and paralysis occur in this disease. The infection may also be seen in flocks without clinical symptoms (Van Empel, 2008; Banani, 2017; Hafez et al., 2020; Noormohammadi, 2021;). One of the economic losses caused by this disease is the elimination of cases in the slaughterhouse (Van Veen *et al*., 2000). The bacterium plays a very important role in respiratory disease complexes, and its different strains have been detected in many countries (Vandamme et al., 1994). The definitive diagnosis of ORT based on clinical symptoms and autopsy findings is difficult and should be done based on the isolation of the bacterium, identification**,** or tracking of antibodies (Shehata & Hafez, 2024; Hafez and Chin, 2020). This research is the first report of ORT infection in quail flocks of West Azarbaijan province, Iran. In addition to isolating the bacteria by microbial culture, the confirmatory diagnosis was performed by the PCR method.

2. Materials and Methods

2.1. Microbiological tests

One hundred thirty (130) samples of tracheal swabs were collected from 13 flocks of quails aged 2 to 4 months that were kept in cages. The swabs were transferred to the tubes containing BHI (Brain Heart Infusion) broth (Merck, Germany). The samples were cultured on agar containing 5% sheep blood and gentamycin (5 µg/mL) and incubated at 37 °C for 24-48 h in a CO_2 incubator. The presumptive colonies (nonhemolytic, small and dewy appearance, and gray to light gray color and morphologically similar to ORT) were used for further confirmatory tests. For purification of colonies, they were cultured on blood agar containing gentamicin (5 µg/mL) and used for Gram staining. Biochemical experiments, such as oxidase, catalase, and growth on McConkey, were performed on the isolates (Hafez and Chin, 2020).

2.2. PCR test

Tracheal swab samples were kept at 4°C until the PCR test was performed on them. Two (2) mL of PBS buffer in each swab sample were transferred to a microtube and centrifuged at 10,000 rpm for 5 min. For lysis of bacteria, cell lysing buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8, 1% SDS, pH = 8) was added to the samples and vortexed for 20-30 s. Then, 30 µL of proteinase K (20 mg/mL) was added to the sample and incubated at 56°C for 30 min. To precipitate the proteins, 450 µL of saturated phenol and 450 µL of chloroform were added to the microtube. The mixture was centrifuged at 12000 rpm for 10 min.

The supernatant of the samples was transferred into new microtube containing cold isopropanol (≥98%) (Sigma-Aldrich, USA). They were centrifuged at 12,000 rpm for 10 min. After discarding the supernatant, pellets were washed with alcohol 70%. The mixture was centrifuged at 14,000 rpm for 5 min. The supernatant was removed, and the resulting DNA was dried for 90 min. Then, 100 µL of distilled water was added to each microtube containing DNA and placed in an incubator at 60 °C for 15 min (Gautam, 2022). To ensure the presence of purified DNA, electrophoresis was performed using 1% agarose gel. To perform the PCR test, the master mix was prepared by including all the tested samples.

The volume of each PCR reaction tube test was 25 µL. Mixed components for each sample included 2.5 µL of 10X buffer, 1.5 µL (25mM) of magnesium chloride ($MgCl_2$), 0.5 µL (10 mM) of dNTP, 0.5 µL (1.25 Unit) of Taq polymerase enzyme, 2 µL of template DNA, 2 µL (10pM) of each primer (forward and reverse) and 14µL of distilled water. To amplify the target gene, a pair of specific reference primers of ORT was used. The sequences of primers were as follows: OR16s-F1: 5′-GAG AAT TAA TTT ACG GAT TAA G-3′ and OR16s-R1: 5′-TTC GCT TGG TCT CCG AAG AT-3′ (Bashashati *et al.,* 2023; yusefi Nejad et al., 2024). The thermal cycling program used for the amplification of the target gene was as follows: initial denaturation for 5 min at 95 °C for one cycle, 35 consecutive cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 90 s, and extension at 72 °C for 90 s, and one cycle of final extension at 72 °C for 10 min.The PCR product was electrophoresed on a 1% agarose gel with a voltage of 80 volts for 45 to 60 minutes. Then the desired gel was observed with DNA-safe stain and UV light.

All animal procedures were approved by the Institutional Animal Care and Use Committee of Islamic Azad university Urmia branch, under approval code IR.IAU.URMIA.1403.140, and were performed in accordance with relevant guidelines and regulations.

3. Results

Bacteria were isolated from 3 out of 13 flocks (23.07%). The isolated bacteria were gram-negative, oxidase-positive, catalase-negative, and lacked growth on agar medium. In the PCR test, the partial 16S rRNA gene (784 bp) was detected in 4 out of 13 flocks (30.76%). No bands were observed in other samples (Fig. 1).

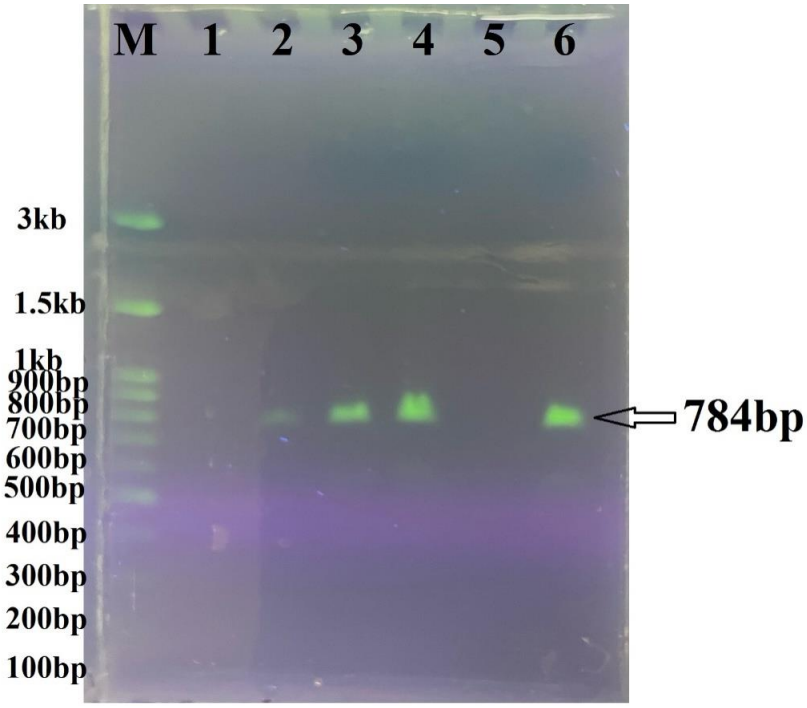

Figure 1. Electrophoresis of the PCR products of the 16S rRNA gene (784 bp) in Ornithobacterium rhinotracheale isolates on 1% agarose gel. M: DNA Ladder 100 bp (Sinaclon, Tehran, Iran), 1: negative control, 2: positive control, 3,4, and 6: positive samples, and 5: negative samples.

4. Discussion

*Ornithobacterium rhinotracheale*is known as a pathogenic agent of birds, which is involved in complex respiratory diseases. The pathogenicity of ORT strains is different. The disease may result in economic losses in the poultry industry. Based on the agar gel precipitation (AGP) test, ELISA, and antigenic characterization, 18 serotypes of ORT have been identified, of which serotype A is the most common serotype (van Empel ,2008; Hafez *et al*,. 2020). The respiratory organs, especially the trachea, lungs, and air sacs, are the best places for the colonization and isolation of ORT. In the present study, sampling was done from the trachea. The bacterium can be isolated in the early stages of infection, but the percentage of isolation cannot be an exact indication of the prevalence rate (Shehata & Hafez, 2024; Hafez and Chin., 2020).

The illness has been documented in chickens and turkeys at a high rate, and the infection primarily affects these two species. The infection has also been reported in other species of birds. However, there are only three reports in this field in Iran. Bashashati et al. (2023) isolated the ORT in flocks of commercial turkeys, quails, and domestic pigeons using bacteriological methods and PCR, including 4 isolates from turkeys, 3 isolates from quails, and 23 isolates from pigeons (Bashashati et al., 2023)

The disease has been reported in chickens and turkeys with a high prevalence, and the

infection is mainly pathogenic in these two species. The infection has also been reported in other species of birds. However, there are only three reports in this field in Iran. Mirzaei *et al.* (2011) isolated the ORT in flocks of commercial turkeys, quails, and domestic pigeons using bacteriological methods and PCR, including 4 isolates from turkeys, 3 isolates from quails, and 23 isolates from pigeons. These isolates showed 98-100% of affinity with related data in the gene bank. Shabani *et al*. (2016) conducted a molecular study on ORT and Newcastle disease virus in 22 ostrich flocks with high losses in Isfahan province (Iran). Their results showed that ORT was not detected in any of the samples. In another study in Isfahan province, the ORT was detected in quails with respiratory symptoms (35%) and without respiratory symptoms and apparently healthy (15%) by PCR test (Asadi *et al*., 2018).

In 2017, for the first time in Iran, ORT was isolated from urine samples of industrial poultry flocks with respiratory symptoms (Banani *et al*, 2000). However, no reports of ORT infection in quails in the West Azerbaijan province have been published. In the present study, for the first time, ORT infection was detected in quail flocks in West Azerbaijan province using microbiological culture (23%) and PCR (30%). Asadpour *et al.* (2014) reported the infection of broiler flocks with ORT in Urmia city (Iran) by microbiological culture (25%), ELISA (75%), and PCR (40%). The correlation between the findings of Asadpour *et al*. (2014) in broilers and the present study in quails proved the prevalence of this infection in West Azerbaijan province, which can be an effective step in epidemiological studies in the region (Asadpour *et al*, 2016). The high affinity of Iranian quail and partridge isolates with native poultry isolates in the phylogenetic analysis of the 16S rRNA gene of ORT in the study of Mirzaei *et al*. (2013) may indicate the same origin of infection between bird species. Also, the prevalence of ORT infection in quail flocks may be due to contamination in chicken and turkey farms and vice versa (Mirzaei *et al*. 2013; Bashashati et al., 2023). Thus, the connection of this hypothesis with the results of the present study and the prevalence of infection in broiler farms in Urmia city by Asadpour *et al*. (2014) shows that there is a possibility of the spread of infection between these two species of birds in this province, which requires phylogenetic studies on the isolates of these two bird species. In other countries, there have been studies on some species of birds except chickens and turkeys. In 2009, the occurrence of otitis and osteomyelitis associated with ORT infection in red-legged partridges (*Alectoris rufa*) was identified using microbiology, hematology, and molecular tests (Moreno *et al*., 2009). It was shown that the genetic characteristics of the isolates were similar (99.9%) and belonged to the same strain.

Welchman showed the role of ORT in connection with the complex respiratory diseases in pheasant flocks in southern England with symptoms of sinusitis, pneumonia, and inflammation of air sacs (Welchman *et al*. 2013). ORT was isolated from the respiratory tract. Also, the 16S rRNA gene sequencing on three isolates showed two distinct genes and serotypes. Hafez *et al*. reported a large outbreak of ORT-induced air sac inflammation in a large farm of falconry. They isolated serotype A from lungs and air sacs (Hafez *et al*., 2010). For the first time in Turkey, Eroksuz *et al*. showed the direct role of ORT infection in the respiratory tract of quails after experimental contamination and the use of various diagnostic techniques (Eroksuz *etal*., 2006). This research was conducted for the first time in West Azarbaijan province to isolate and identify ORT in quail flocks through culture and PCR. In the present study, ORT was isolated from quails without clinical respiratory symptoms. This finding indicates that although ORT is known as a pathogen, it can also be present in the respiratory tracts of healthy birds, causing infection in predisposing conditions such as secondary viral or bacterial infections.

Considering the high percentage of bacterial identification in molecular tests compared to culture methods, it can be concluded that the PCR test can be effective in identifying bacteria even when the bacteria are dead. On the other hand, with a lower probability, it can be justified that the bacteria have lost their activity during the transfer of the samples to the laboratory. This is consistent with the results of the study by Mirzaei *et al*. (2011), who detected the ORT infection in 1.2% of quails in tracheal swab culture and 50% of tissue samples in the PCR test. These results indicate the greater validity of the PCR test compared to culture methods (Bashashati et al., 2023).

Conclusion

The present research proved the presence of ORT infection in quail flocks in West Azarbaijan province and showed that PCR is a more reliable method for the diagnosis of ORT. On the other hand, the evaluation of vaccination performance in quail parent flocks should be considered to control the disease in quail farms and its spread to other poultry flocks. However, to make a better judgment, more detailed investigations on quail flocks suffering from respiratory diseases seem to be necessary.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Refereces

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