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Short communication

PREVALENCE OF *ORNITHOBACTERIUM RHINOTRACHEALE* AT BROILER CHICKEN FARMS IN SOUTHWEST IRAN

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Summary

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Ornithobacterium rhinotracheale (*ORT*) is a Gram negative, pleomorphic, rod-shaped and non-motile bacterial pathogen most known for causing respiratory tract infections in chickens. Ornithobacteriosis has been reported in almost all countries around the world. The objective of this study was to determine the prevalence of *ORT* in broiler flocks in southwest Iran. DNA was extracted from 230 collected tracheal swabs and lungs samples and amplified by *ORT 16S rRNA* gene specific primers using the PCR technique. *ORT* DNA was detected in 63 broiler chickens samples (27.39%). The results of this study demonstrate the wide spread of *ORT* in broiler chickens and confirmed that infection with *ORT* was highly prevalent in southwest Iran.

Key words: broiler chickens**,** *Ornithobacterium rhinotracheale* (*ORT*), PCR

Ornithobacterium rhinotracheale (*ORT*) is a Gram negative, pleomorphic, rod-shaped non-motile bacterial pathogen (Ghanbarpour *et al.*, 2009). *ORT* is mostly regarded as a facultative pathogen and in field cases; simultaneous isolation of *ORT*, respiratory viruses and/or other bacteria is frequently encountered (Marien *et al.*, 2006). So far, no special structures or properties, such as pili, fimbriae, plasmids or specific toxic activities, are known to exist within the species (Rahimi *et al.*, 2007), but 12 different *ORT* serotypes (A–L) have been reported (Banani *et al.*, 2001). The name *Ornithobacterium* was suggested for the new genera within the rRNA super family and the name *rhinotracheale* for the species (Koga *et al.*, 2005). The first recorded isolation of *ORT* was made from turkeys in Germany in 1981 (Banani *et al.*, 2001). *ORT* has been isolated from chickens, chukar partridges, ducks, geese, guinea fowls, gulls, ostriches, partridges, pheasants, pigeons, quails, rooks and turkeys (Allymehr, 2006). This bacterium causes respiratory tract infections in birds all over the world (Schuijffel *et al.*, 2005; Ghanbarpour *et al.*, 2009). Disease associated with *ORT* was reported for the first time in the states of Minnesota and Wisconsin (Amonsin *et al.*, 1997). *ORT*

was detected in meat turkeys and broilers in South Africa, Germany, the United States, France, and the Netherlands (Empel *et al.*, 1997) and has been incriminated as a possible additional causative agent in the respiratory disease complex (Ak *et al.*, 2001).

The disease typically appears in birds 11 to 26 weeks of age, with mortality rates ranging from 3 to 7%. It is not known whether the disease outbreaks resulted from the dissemination of a single or multiple clones (Amonsin *et al.*, 1997). *ORT* can be a primary or secondary etiological agent depending on strain virulence, adverse environmental elements, immune condition of the flock, and presence of other contagious agents (Suzuki *et al.*, 2010). Clinical signs associated with *ORT* infection include tracheitis, airsacculitis, pericarditis, sinusitis, and exudative pneumonia. At *post mortem* examination, the most striking feature was foamy white, yoghurt-like exudate in the air sacs although pneumonia was also found (Banani *et al.*, 2001). Other *post mortem* lesions associated with *O. rhinotracheale* infection identified were fibrin purulent pneumonia, airsacculitis, peritonitis with foamy exudates and arthritis (Rahimi *et al.*, 2007). Respiratory problems, together with purulent pneumonia, airsacculitis, severe growth retardation, and rapidly increasing mortality, were reported in chickens in several areas in world (Empel *et al.*, 1997). In some cases, chickens infected with *ORT* did not present with clinical signs (Zain *et al.*, 2008). Disease caused by *ORT* may be reduced by preventing predisposing factors including inadequate ventilation, high ammonia levels, too high or too low relative humidity and infection with additional pathogenic agents (Marien *et al.*, 2006).

Respiratory tract infection is a major problem within the poultry industry and is accompanied by substantial economic losses due to retarded growth, increased medication costs, high culling rates and poor production (Ghanbarpour *et al.*, 2009). Although microbiological isolation and identification have been done by several investigators, there are few reports using molecular identification techniques such as polymerase chain reaction (PCR) and 16S ribosomal gene sequencing (Koga *et al.*, 2005). Because ORT is difficult to identify, the use of a reliable identification method is essential. PCR assays were shown to be useful for identification purposes (Ozbey *et al.*, 2004). The aim of this research was to determine the prevalence of *ORT* among the broiler chickens in southwest Iran using *16S rRNA* gene.

In summer 2010, tracheal swabs and lungs from 230 broilers were collected at slaughterhouses in two provinces (95 samples from Chaharmahal Va Bakhtiari province and 135 samples from Isfahan province) in southwest Iran. Samples originated from 63 flocks reared in 25 farms.

ORT genomic DNA was extracted from swabs and lungs samples using DNA extraction kit (QIAGEN Ltd., Crawley, UK) according to manufacturer's instructions.

In this study, primers were designed according to the published sequence for *16S ribosomal RNA* gene of *Ornithobacterium rhinotracheale* (accession number: U87106). The primers pairs sequences were as followed: the forward primer was Ornitho-F: 5'-TGGCATCGATTAAAAT TGAAAG-3' and the reverse primer was Ornitho-R: 5'-CATCGTTTACTGCGTG GACTAC-3'. These primers amplify a 625 bp fragment after PCR reaction.

PCR was performed in a final volume of 25 μL reaction buffer containing 20 ng

genomic DNA, 2 mM $MgCl_2$, 200 mM dNTP mix, 2.5 µL of 10×PCR buffer, 25 pmol of each primer (Ornitho-F and Ornitho-R), and 1 unit of *Taq* DNA polymerase (Roche Applied Science). Reaction mixtures were preincubated at 94°C for 5 min, followed by 32 cycles at 94°C for 1 min, 61°C for 1 min, 72 °C for 1 min and a final extension step at 72 °C for 5 min. The PCR product was analyzed by electrophoresis in 1% agarose gel in 1× TBE buffer and visualized by ethidium bromide staining on an UV transilluminator. The molecular size of PCR products were compared with a 100 bp DNA ladder (Fermentas, Germany).

Genomic DNA was successfully extracted from chicken samples using the DNA extraction kit. The quality of the extracted DNA was investigated using spectrophotometric absorbance ratios at 260 nm and it had a sufficient quality for PCR amplification. Analysis of PCR products of *16S ribosomal RNA* gene of *Ornithobacterium rhinotracheale* on agarose gel revealed a 625 bp fragment (Fig. 1). Out of 230 chicken samples (Table 1) examined for the presence of *ORT* DNA, the prevalence of *ORT* in the two provinces studied was 27.39% (63 samples).

The first documented isolation and identification of *ORT* in Iran was made from 4-week-old broiler sin 2000 (Banani *et al.*, 2000).

Suzuki *et al.* (2010) used a commercial ELISA for the detection of antibodies against *ORT* in chicken serum and reported a flock-level apparent prevalence and true prevalence of 30% and 17%, respectively. Allymehr (2006) indicated that the prevalence of *ORT* antibody was 92% in the broiler and broiler breeder flocks in West Azerbaijan province (northwest Iran). Ozbey *et al.* (2004) detected antibodies against *ORT* in 33 (10.2%) of the 324 sera analyzed by ELISA and a 784 bp fragment of the *16S rRNA* gene was amplified using specific primers in the PCR. Also, all *ORT* isolates that were positive by culture were also detected to be positive by the PCR. Koga *et al.* (2005) indicated that from the original 75 strains isolated from 75 clinical samples from which *ORT* was recovered during 1998-2000 in Peru, 25 were selected for further study based on *ORT* as the primary pathogenic isolate. All 25 strains of *ORT* tested with rep-PCR had a genetic profile similar to that of *ORT* American Type Culture Collection

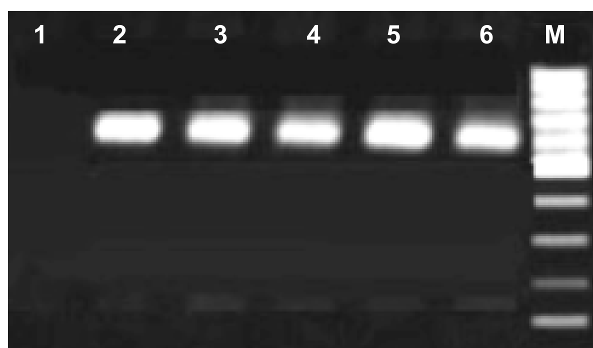

Fig. 1. Agarose gel stained with ethidium bromide, with PCR products of *Ornithobacterium rhinotracheale* (ORT) isolates. Lane M: 100 bp DNA ladder, lane 1: negative control, lane 2: positive control, lanes 3–6: *ORT* positive samples.

Table 1. Prevalence of *Ornithobacterium rhinotracheale* at chicken farms in two provinces of Iran

Province	Number of samples	ORT-negative, number (%)	ORT-positive, number (%)
Chaharmahal Va Bakhtiari	95	62 (65.27%)	33 (34.73%)
Isfahan	135	105 (77.78%)	30 (22.22%)
Total	230	167 (72.61%)	63 (27.39%)

51463, indicating the presence of only one genotype in the *ORT* strains studied (Koga *et al.*, 2005). In 2009, *ORT* prevalence was studied in broiler flocks in southeast Iran and strains were identified in 42.9% of samples (Ghanbarpour *et al.*, 2000). In June 2005, another research team has isolated *ORT* in the chickens of a large broiler farm in Kermanshah province, west Iran in the serum plate agglutination (SPA) test and reported a prevalence of 13.6% (Rahimi *et al.*, 2007).

The serotype specificity of the ELISA is a disadvantage, although a number of commercial ELISA have been described as highly sensitive to various *ORT* serotypes. As *ORT* has an economical importance for the poultry industry, its prevalence has to be considered in programmes for prevention and control of poultry respiratory diseases, especially during the cold seasons when it occurs at the highest rate (Allymehr, 2006).

According to high transmission of *ORT* infection in cold weather, the present study was performed in fall and winter seasons. There are many industrial and native turkey breeding centres in southwest of Iran and the turkey is the main host of *ORT* infection in this area. It seems that the existence of turkeys in this region is causing the high prevalence of *ORT* infection in broiler chicken. Furthermore, the studied region is the transition route for transportation of food, meat and other bird products between the central and south parts of Iran. So, the high prevalence of *ORT* infection in turkeys could cause its transmission to other domestic birds such as broiler chickens and entail economic and health damages to the industrial poultry breeding in this area.

In conclusion, the results of the present study demonstrated a high spread of *Ornithobacterium rhinotracheale* infection among broiler chickens in southwest Iran. The control of this microorganism is useful for prevention and reduction of the incidence of ornithobacteriosis.

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