Prevalence of *Ornithobacterium rhinotracheale* at broiler chicken farms in southwest Iran

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Summary


*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 (Schuijf *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, airsaccuitis, 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, airsaccuitis, peritonitis with foamy exudates and arthritis (Rahimi et al., 2007). Respiratory problems, together with purulent pneumonia, airsaccuitis, severe growth retardation, and rapidly increasing mortality, were reported in chickens in several areas in the 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 MgCl₂, 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 in 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). Ozbeay 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.
Prevalence of Ornithobacterium rhinotracheale at broiler chicken farms in southwest Iran

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

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of samples</th>
<th>ORT-negative, number (%)</th>
<th>ORT-positive, number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaharmahal Va Bakhtiari</td>
<td>95</td>
<td>62 (65.27%)</td>
<td>33 (34.73%)</td>
</tr>
<tr>
<td>Isfahan</td>
<td>135</td>
<td>105 (77.78%)</td>
<td>30 (22.22%)</td>
</tr>
<tr>
<td>Total</td>
<td>230</td>
<td>167 (72.61%)</td>
<td>63 (27.39%)</td>
</tr>
</tbody>
</table>

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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Paper received 17.12.2010; accepted for publication 08.04.2011

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