Ornithobacterium rhinotracheale and Mycoplasma synoviae in broiler chickens in Jordan

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Summary
A cross-sectional study was conducted from November 2008 to July 2010 in commercial broiler flocks in southern (n = 50) and northern (n = 50) areas of Jordan, to determine the flock-level prevalence of Ornithobacterium rhinotracheale (ORT) and Mycoplasma synoviae (MS) infections. Tracheal swabs were collected from commercial broilers with respiratory disease and tested by polymerase chain reaction. In total, 21% (95% CI: 18–45%) and 25% (95% CI: 20–51%) of commercial broiler flocks were positive for ORT and MS, respectively. In the southern areas the prevalence of flocks with positive tracheal swabs for ORT and MS was 16% and 10%; in the northern areas the prevalence was 26% and 40%, respectively. Of the flocks tested, 7% were infected with ORT and MS simultaneously. Further epidemiological studies are recommended to determine risk factors and evaluate the economic consequences of ORT and MS infections in the region. Furthermore, studies are required to isolate ORT and MS and develop vaccines against the local field isolates.

Keywords

Introduction

Ornithobacterium rhinotracheale (ORT) is a recently described species of bacterium (24) associated with respiratory disease, growth retardation, mortality and decreased egg production in chickens and turkeys (7). It can cause a highly contagious disease in poultry, although the severity of clinical signs, duration of the disease and mortality have been found to be extremely variable (23). The bacterium has been isolated from chickens, turkeys, chukar partridges, quail, ducks, geese, ostriches and guinea fowl. It can be a primary or secondary aetiological agent, and this depends on strain virulence, adverse environmental factors, the immune status of the flock, and the presence of other infectious agents (23).

Mycoplasma synoviae (MS) is an important pathogen of domestic poultry, and causes economic losses to the poultry industry (14). The agent most frequently causes a subclinical upper respiratory infection, which can progress to respiratory disease with air sac lesions when exacerbated by other respiratory pathogens (e.g. Newcastle disease virus, infectious bronchitis virus) or when more virulent strains of MS are involved (15, 17). The infection can also become systemic and this results in an acute or chronic infectious synovitis (14).
In Jordan, where the poultry industry is of great importance both socially and economically, disease prevention programmes are crucial to avoid virus spread and the development of disease (12). Respiratory diseases in chickens in Jordan are a major concern, because they cause catastrophic economic losses to the poultry industry. No previous studies have been carried out in Jordan to determine the involvement of ORT and MS in respiratory disease in broiler chicken flocks. The aim of the present study was to estimate the flock-level prevalence of ORT and MS infections in broiler flocks located in the southern and northern areas of Jordan, using polymerase chain reaction (PCR) on tracheal swab samples.

**Materials and methods**

**Broiler flocks**

This study, which was carried out between November 2008 and July 2010, involved the examination of 100 commercial broiler chicken flocks, located in the southern (n = 50) and in the northern (n = 50) areas of Jordan, in which chickens were identified as suffering from respiratory disease. The chickens were kept until they were 40 to 45 days old and then sent for processing. The broiler chicken vaccination programme usually involved two live vaccines against Newcastle disease virus and infectious bronchitis virus, administered either by spray or in the drinking water at 1 and at 11 to 12 days old, and one infectious bursal disease vaccine (mild or moderate strain) administrated in the drinking water at 12 days old.

The southern and northern areas of Jordan are the most important parts of the country for the poultry industry. Taken together, these areas contain 170 broiler-type chicken farms, with an average of 10,000 to 20,000 chickens per farm (12). The flocks were selected with the help of the Ministry of Agriculture, which maintains records of the name of the owner and the location of each broiler flock in these regions. In the majority of flocks, signs of respiratory disease usually appeared in chickens of 29 to 34 days old; six chickens from each flock were taken during the acute phase of disease and sent to the Provimi Jordan laboratory. Affected chickens suffered from nasal and ocular discharge and conjunctivitis, as well as being weak and reluctant to move. Gross lesions observed in these flocks included a mild congestion of the trachea and mild airsacculitis. In the laboratory, samples of tracheae from each flock were collected and stored at -80°C until used for DNA extraction. None of these flocks was vaccinated against MS or ORT.

**Deoxyribonucleic acid extraction**

Frozen tracheas from each flock were swabbled with sterile swabs (Heinz Herenz Medizin GmbH, Hamburg, Germany). The swabs were placed in 1,000 µl phosphate-buffered saline (PBS; pH 7.2) and were scraped on the side of the tube to facilitate removal of the contents from the swab head. Bacterial DNA was extracted and purified using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s recommended procedure.

**Molecular polymerase chain reaction to detect Ornithobacterium rhinotracheale and Mycoplasma synoviae**

The primers used in this study for ORT consisted of the following sequences: OR16S-F1 (5′-GAG AAT TAA ACG GAT TAA G-3′) and OR16S-R1 (5′-TTC GCT TGG TCT CCG AA GAT-3′) (Alpha DNA, Montreal, QC, Canada), which amplify a 784-base pair (bp) fragment of the 16S ribosomal ribonucleic acid (rRNA) gene of ORT but not of other closely related bacteria (11). The primers used for MS consisted of the following sequences: MS-F (5′-GAG CAA AAT AGT CAT ATC A-3′) and MS-R (5′-CAG TGG TCT CCG AAG TTA ACA A-3′) (Alpha DNA, Montreal, QC, Canada), which are complementary to the 16S rRNA gene of MS, and the PCR produces a 214-bp fragment in positive samples (16). The PCR mix was prepared in a volume of 50 µl containing 25 µl of master mix (Taq polymerase 5 U/µl, 400 mM of deoxynucleotide triphosphate mixture, and 3 mM of MgCl₂), 18 µl of nuclease-free water (Promega Corp., Madison, WI, USA), 1 µl (50 pmol/µl) of each oligonucleotide, and 5 µl of DNA template. The samples were amplified under the following conditions and the reaction was carried out in a DNA Engine® thermal cycler (Bio-Rad Laboratories Ltd, Mississauga, ON, Canada). For each flock there were two PCR tubes, one tube for ORT and one for MS, which were included in the same run with the same programme but different annealing temperatures (the PCR tubes for ORT and MS were placed in specific wells according to the annealing temperature). The temperature gradient programme for one cycle consisted of 3 min at 94°C, then 35 cycles at 94°C for 2 min (denaturation), 58°C for 1 min (for ORT), 54°C for 1 min (for MS; annealing), and 72°C for 2 min (extension). Ornithobacterium rhinotracheale vaccine Nobilis OR inac (Intervet, Wim de Körverstraat, Mississauga, ON, Canada) and MS® (Bioproperties, Australia) were used as positive controls for DNA extraction and PCR. A negative control (nuclease-free water) was also used in each run.

**Agarose gel electrophoresis**

Polymerase chain reaction products were electrophoresed on a 2% agarose gel in Tris-acetate-EDTA buffer (40 mM of Tris and 2 mM of ethylene-diamine-tetra-acetic acid [EDTA], pH 8.0) containing ethidium bromide (Promega Corp., Madison, WI, USA) for 45 min at 100 V and
visualised under UV light (Alphalma, Alphalnotech, San Leandro, CA, USA).

**Statistical analysis**

Confidence limits (95%) for prevalence were calculated using an approximation to the standard normal distribution. The unit of statistical analysis was the flock. Comparison of prevalence between the two areas of Jordan was done using the chi-square test, and p-values of ≤0.05 were considered statistically significant. All analysis was performed using SAS Version 6.0 (20).

**Results**

Samples from a total of 100 broiler flocks with a history of respiratory disease were received by the Provimi Jordan laboratory from southern (n = 50) and northern (n = 50) areas in Jordan and tested by PCR for the presence of ORT and MS in tracheal samples. Of the 100 flocks studied, 21% (95% CI: 18–45%) and 25% (95% CI: 20–51%) were positive for ORT and MS infections, respectively. The distribution of flocks in the southern and northern areas of Jordan that were positive by PCR is shown in Table I. In the southern areas the prevalence of flocks with positive tracheal swabs for ORT and MS was 16% and 10%, respectively; in the northern areas the prevalence was 26% and 40%, respectively; the difference between the regions was statistically different (X^2 = 4.94, d.f. = 1, p = 0.0263). In addition, 7% of the 100 flocks tested were infected with ORT and MS simultaneously.

**Discussion**

Respiratory tract infection is a major problem in the poultry industry throughout the world. It has a major economic impact as a result of treatment costs and losses caused by the deaths of animals and higher rates of carcass condemnation. Respiratory diseases in poultry arise from various causes, including bacteria (5), viruses (2) and fungi (1).

In the current study, ORT and MS were detected by PCR in 21 and 25 of the flocks tested, respectively (Table I). Given that all the birds tested in this study were older than four weeks of age, were suffering from respiratory disease, and were not vaccinated against MS and ORT, these data suggest that the infected flocks were exposed in the field to MS and/or ORT. Respiratory diseases in poultry have been reported to be caused by mixed or single infections with several agents (18, 19, 25, 27). In the current study, 7% of the flocks tested were infected with ORT and MS at the same time.

**Ornithobacterium rhinotracheale** infections have been reported in many countries around the world, including Belgium (26), Canada (13), the United States (6), Israel (4), the Republic of Korea (28), Japan (22), Egypt (8), Turkey (10), Chinese Taipei (21) and Iran (3). In Jordan, ORT was first isolated from broilers in 2001 (9). In the current study, ORT was detected in 21 out of the 100 flocks tested. Of these 100 flocks, 14% were diagnosed to be infected with ORT alone, whereas in the other 7% of flocks ORT was combined with MS (see Table I). This study and previous reports (9) have confirmed the endemic nature of ORT infection in Jordan.

**Table I**

<table>
<thead>
<tr>
<th>Area</th>
<th>Agent</th>
<th>PCR-positive flocks</th>
<th>PCR-negative flocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
<td>Number</td>
</tr>
<tr>
<td>Southern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORT alone</td>
<td>6</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>MS alone</td>
<td>3</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>MS + ORT</td>
<td>2</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>ORT total</td>
<td>8</td>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>MS total</td>
<td>5</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Northern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORT alone</td>
<td>8</td>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>MS alone</td>
<td>15</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>MS + ORT</td>
<td>5</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>ORT total</td>
<td>13</td>
<td>26</td>
<td>37</td>
</tr>
<tr>
<td>MS total</td>
<td>20</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

MS: *Mycoplasma synoviae*  
ORT: *Ornithobacterium rhinotracheale*  
PCR: Polymerase chain reaction
Mycoplasma synoviae was detected in 25 of the 100 flocks tested. Eighteen percent were diagnosed to be infected with MS alone, whereas in the other infected flocks MS was combined with ORT (Table I). Mycoplasma synoviae has been reported to occur throughout the world (14). This is the first report of the involvement of MS in respiratory disease seen in broiler flocks in Jordan, and it will pave the way for further studies of MS.

The high prevalence of both pathogens in the northern area in comparison with the southern area may be attributed to the diversity of the chicken population (layers, breeders and broilers) and the presence of a large number of broiler farms in the northern area in comparison with the southern area.

Conclusion

This study has shown that ORT and MS are involved in respiratory disease in broiler chickens in Jordan. Further studies are necessary to assess the circulating strains, the economic losses caused by infections and co-infections of MS and ORT, and the costs and benefits of countermeasures. Furthermore, studies are required to isolate ORT and MS and to develop vaccines against the local field isolates. In addition, farmers need to be educated about the signs of infection and the importance of these pathogens.

Acknowledgements

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Infections à *Ornithobacterium rhinotracheale* et à *Mycoplasma synoviae* chez des poulets de chair en Jordanie


Résumé

Une étude transversale a été conduite de novembre 2008 à juillet 2010 dans des élevages commerciaux de poulets de chair dans des zones situées au sud (n = 50) et au nord (n = 50) de la Jordanie afin de déterminer la prévalence au niveau des élevages des infections à *Ornithobacterium rhinotracheale* et à *Mycoplasma synoviae*. Des écouvillons trachéaux prélevés chez des volailles commerciales présentant des troubles respiratoires ont été soumis au test d’amplification en chaîne par polymérase (PCR). Les résultats ont été positifs pour *O. rhinotracheale* et *M. synoviae* dans respectivement 21 % (intervalle de confiance [IC] à 95 % compris entre 18 % et 45 %) et 25 % (IC à 95 % compris entre 20 % et 51 %) des élevages commerciaux de poulets de chair.
La prévalence des élevages possédant des volailles dont les écouvillons trachéaux se sont révélés positifs pour *O. rhinotracheale* et *M. synoviae* était respectivement de 16 % et de 10 % dans les zones du Sud couvertes par l’étude, et de 26 % et 40 % dans les zones du Nord. Parmi les élevages analysés, 7 % présentaient une infection concomitante à *O. rhinotracheale* et à *M. synoviae*. Des études épidémiologiques plus approfondies permettront d’élucider les facteurs de risque et d’évaluer les conséquences économiques de ces infections dans la région. En outre, des études devront être conduites pour isoler *O. rhinotracheale* et *M. synoviae* et pour mettre au point des vaccins adaptés contre les souches de terrain isolées localement.

**Mots-clés**

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**Ornithobacterium rhinotracheale y Mycoplasma synoviae en pollos asaderos de Jordania**


**Resumen**
Los autores describen un estudio transversal realizado entre noviembre de 2008 y julio de 2010 con pollos asaderos de granjas industriales de las zonas meridional (*n* = 50) y septentrional (*n* = 50) de Jordania con objeto de determinar la prevalencia en las bandadas de las infecciones por *Ornithobacterium rhinotracheale* (ORT) y *Mycoplasma synoviae* (MS). Tras extraer hisopados tráqueos de pollos con afección respiratoria, se analizaron esas muestras por reacción en cadena de la polimerasa (PCR). En total, un 21% (IC 95%: 18–45%) y un 25% (IC 95%: 20–51%) de las bandadas industriales resultaron positivas para ORT y MS, respectivamente. En las zonas meridionales, la prevalencia de bandadas con muestras tráqueas positivas para ORT y MS fue del 16% y el 10%, respectivamente; en las zonas septentrionales, esos porcentajes fueron del 26% y el 40%. De las bandadas analizadas, un 7% estaban infectadas a la vez por ORT y MS. Los autores recomiendan que se efectúen nuevos estudios epidemiológicos para determinar los factores de riesgo y evaluar las consecuencias económicas de ambas infecciones en la región. También se precisan estudios que permitan aislar ambos microorganismos y elaborar vacunas contra las cepas locales aisladas.

**Palabras clave**
References


