

Experimental Dual Challenge with *Ornithobacterium rhinotracheale* and *Mycoplasma synoviae* in Broilers

R. Cerdá^{AB}, J. Uriarte^A, J. Origlia^A and M. Petrucci^A

^ALaboratory of Diagnosis of Avian and Piliferou Diseases. Faculty of Veterinary Medicine. National University of La Plata. CC296. 1900. La Plata. Argentina.

^BECO Animal Health, London, UK.

SUMMARY. *Ornithobacterium rhinotracheale* (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens. Nevertheless, there was no demonstrated their pathogenic synergism in experimental dual infections in broiler chickens. In the present study, three week old broiler chickens were inoculated either with a standard ORT strain (intratracheally), a MS field strain (aerosol) or both agents at the same time. At 7 and 14 days post challenge chickens from each group were euthanatized and analyzed for thoracic and abdominal air sac lesions as well as lung and trachea lesions. Significant differences in score lesions were seen in group ORT/MS comparing with the uninoculated control group and MS group. Infections were confirmed by strain re-isolation, PCR and serology tests. This is the first report of an ORT/MS dual infection in chickens.

Key words: broilers, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale*

Abbreviations: CCU = colony-changing-units; CFU = colony-forming units; IB = Infectious Bronchitis; ND = Newcastle Disease; PI = post inoculation

INTRODUCTION

Ornithobacterium rhinotracheale (ORT) and *Mycoplasma synoviae* (MS) have been recognized as primary respiratory pathogens in broiler chickens (6). Both bacteria species have a worldwide distribution and are associated to respiratory disease and high economic losses (3, 4). Despite it is well known the multifactorial etiology of the respiratory complex in poultry, only a few experimental trials have been done to establish possible mutual interactions between bacteria and virus with ORT (1). Besides one report regarding field dual ORT and MS infection in turkeys (7), there are no reports on natural or experimental ORT and MS interaction in broiler chickens. The present study was undertaken to evaluate the possible synergism between ORT and MS in broiler chickens.

MATERIALS AND METHODS

Experimental Animals. Forty commercial Ross broiler chickens free of mycoplasma and salmonella were housed in isolators after hatching with food and water *ad libitum*.

***Ornithobacterium rhinotracheale* inoculum.** The ORT challenge inoculum was prepared from strain ATCC51463. The bacterium was cultured in 5 % sheep blood agar media supplemented with 10 ug/ml gentamicine in a 5 % CO₂ atmosphere. After 48 hs incubation ten colonies were transferred into 5 ml of PBS and a challenge inoculum suspension containing 10⁹ colony forming units (CFU)/ml was prepared.

***Mycoplasma synoviae* inoculum.** The MS strain used was a recent field isolate from an outbreak of infectious synovitis in a laying hen farm from Buenos Aires province, Argentina. The strain was cloned and prepared using Frey (Gibco) broth supplemented with 12% pig serum and resulted in an organism density of 10^8 color changing units/ml (CCU/ml).

Experimental design. Forty chickens were randomly divided into 4 experimental groups of 10 birds at 1 day of age. By 2 weeks of age the birds showed to be free of maternally derived antibodies to ORT and MS by a commercial enzyme-linked immunosorbent assay (ELISA)(IDEXX) and rapid serum agglutination test (RSA)(Intervet, The Netherlands), respectively. At the same time all the chickens received a ND-BI (La Sota-Masachusetts) vaccination via eye drop. At three weeks of age the different groups received the following treatments: (ORT/MS) challenge with ORT and MS cultures, (MS) MS culture, (ORT) ORT culture and an uninoculated control group.

Parameters of infection. Postmortem gross lesions were analyzed at 7 and 14 days postinoculation (PI) in 5 chickens from each group after necropsy. Thoracic and abdominal air sac as well as lung and trachea lesions were scored by a scoring system. Air sacs were examined and given a score according to the amount of cheesy exudate contained within the air sacs as follows: 0 = no visible exudate; 1 = 25% or less of air sac contained exudate; 2 = 25 to 50% of air sac contained exudate; 3 = more than 50% of air sac contained exudate. The maximum score per bird = 6; lungs, 0 = no abnormalities, 1 = unilateral pneumonia, 2 = bilateral pneumonia. The maximum score per bird = 2; trachea, 0 = no abnormalities, 1 = some exudate in the tracheal lumen, 2 = lumen of the trachea filled with exudate. The maximum score per bird = 2.

Confirmation of the infection. To confirm the experimental infection by ORT and MS cultures, tracheal pool swabs for strain re-isolation and PCR as well as blood for serological tests, RSA test for MS and ELISA test for ORT, were taken at the end of the trial.

Statistical analysis. The statistical analyses for the pathologic lesions were performed by the Kruskal-Wallis one-way analysis of variance.

RESULTS

The lesion score results are shown in the table. Significant differences in score lesions ($P < 0.05$) were only seen between group ORT/MS comparing with group MS in air sac lesions at 7 and 14 days PI and with the uninoculated control group in all the organs evaluated and at both times of necropsy. Infections were confirmed by strain re-isolation (100% for MS and 30% for ORT), PCR (100% for both agents) and serology tests (100% for both agents).

DISCUSSION

Although no interaction have been seen between ORT and MS in turkeys under field conditions (7), a high synergism between both agents has been seen under the experimental conditions of the present study in broilers. The trigger with the ND-IB vaccine and the pathogenicity of the MS field strain used could be the reason of such results. The Argentinean MS field strain used has shown to

be high pathogenic for boilers (2). Further studies should be carried with different MS strains and different management conditions in order to know the possible results according to the different scenarios in the field.

REFERENCES

1. Marien, M., A. Decostere, A. Martel, K. Chiers, R. Froyman and H. Nauwynck. Synergy between avian pneumovirus and *Ornithobacterium rhinotracheale* in turkeys. *Avian Pathol.* 34 (3), 204-211. 2005.
2. Meghan, M., S. Kleven and D. Brown. Sialidase Activity in *Mycoplasma synoviae*. *Avian Dis.* 51:829–833. 2007.
3. Kleven, S. *Mycoplasma synoviae* infection. In: *Diseases of Poultry* (Calnek, B. W., Ed.) 10th edition, Iowa State University Press, Ames, Iowa, pp. 220–225. 1997.
4. van Empel, P. and H. Hafez. *Ornithobacterium rhinotracheale*: a review. *Avian Pathol.* 28. 217-227. 1999.
5. van Veen, L. Country report on The Netherlands. In: *Aerosols, Newsl. World Vet. Poult. Assoc.* p. 12. 1999.
6. van Veen, L., P. van Empel and T. Fabri. *Ornithobacterium rhinotracheale*, a primary pathogen in broilers. *Avian Dis.* 44. 896-900. 2000.
7. Zorman-Rojs, O., I. Zdovc, D. Bencina and I. Mrzel. Infection of Turkeys with *Ornithobacterium rhinotracheale* and *Mycoplasma synoviae*. *Avian Dis.* 44:1017-1022. 2000.

ACKNOWLEDGMENTS

We thank Fernando Marino and Daniel Gornatti for technical assistance. This study was primarily supported by ECO Animal Health, UK. We acknowledge Dr Martin Cardaci (Vetanco) for MS strain field finding and collection.

Table: Postmortem lesion scores after 7 and 14 days post-inoculation of ORT and MS.

| Group | 7 days PI | | | 14 days PI | | |
|--------------|--------------------------|------------------------|------------------------|--------------------------|------------------------|-----------------------|
| | Air sacs | Lung | Trachea | Air sacs | Lung | Trachea |
| ORT/MS | 5.4 (90.0) ^b | 1.8 (90) ^b | 1.8 (90) ^b | 3.6 (60.0) ^b | 1.4 (70) ^b | 1.4 (70) ^b |
| MS | 2 (33.3) ^a | 0.2 (10) ^a | 1 (50) ^{ab} | 2.4 (40.0) ^a | 0.4 (20) ^{ab} | 1.2 (60) ^b |
| ORT | 2.2 (36.7) ^{ab} | 0.6 (30) ^{ab} | 0.8 (40) ^{ab} | 2.2 (36.7) ^{ab} | 0.6 (30) ^{ab} | 1 (50) ^{ab} |
| Uninoculated | 0.2 (3.3) ^a | 0.2 (10) ^a | 0.2 (10) ^a | 0.4 (6.7) ^a | 0.2 (10) ^a | 0.2 (10) ^a |

Scores are given as the maximum possible lesion scores in the group (percentage in brackets). Within columns, values with different lowercase superscripts are significantly different ($P < 0.05$).