CASE REPORT

Apparent eradication of *Mycoplasma synoviae* in broiler breeders subjected to intensive antibiotic treatment directed to control *Escherichia coli*

Laurimar Fiorentin¹*, Ricardo A. Soncini², José Luiz A. da Costa², Marcos A. Z. Mores², Iara M. Trevisol², Márcia Toda² and Nilson D. Vieira¹

¹Brazilian Agricultural Research Corporation—EMBRAPA, Embrapa Suínos e Aves, BR 153, km 110, Vila Tamanduá, Caixa Postal 21, 89700-000, Concórdia, SC, Brazil, ²Sadia SA, Rua Senador Atílio Fontana 86, 89700-000, Concórdia, SC, Brazil

A *Mycoplasma synoviae* (MS)-free flock of broiler breeders was housed for brooding and rearing on an MS endemic farm. PCR revealed that the flock became infected within nine weeks. At 22 weeks the flock was transferred to a clean and disinfected house on a previously depopulated farm. The birds were then subjected to three treatments with fluoroquinolones due to recurrent *Escherichia coli* peritonitis and from the 32 weeks of age they received 600 ppm of oxytetracycline hydrochloride continuously in the feed. Monitoring by PCR showed a decrease in MS positive birds after 34 weeks of age and MS may have been eradicated as judged by consistent negative results in PCR. We conclude that intensive antibiotic treatments supported by adequate biosecurity could clear MS from infected broiler breeders.

Introduction

*Mycoplasma synoviae* (MS) infection is usually asymptomatic in broiler breeders in Brazil but the possibility that it might play an important role in complex respiratory disease in the offspring has motivated breeding companies to consider eradication. Broiler breeders are usually maintained under high biosecurity while broilers are often reared in areas where infectious bronchitis is endemic and *Escherichia coli* is a constant threat.

In the past, mycoplasma eradication programmes have been based on antibiotic or heat treatment of fertile eggs. Mycoplasmas are sensitive to a number of different antibiotics including tetracyclines (Cerda et al., 2002; Chopra & Roberts, 2001) and fluoroquinolones (Cerda et al., 2002; Hannan et al., 1997; Jordan et al., 1998; Wellehan et al., 2001). These are broad spectrum antibiotics and can be used to control a wide range of bacteria including *E. coli*. Here we report the fortuitous finding that three in-feed treatments of broiler breeders with fluoroquinolones, coupled with treatment with oxytetracycline, which were aimed at controlling recurrent *E. coli* peritonitis, also resulted in apparent eradication of MS from the flock.

Material and Methods

Source of broiler breeders

Broiler breeders were obtained from an MS- and *Mycoplasma gallisepticum* - (MG) free primary breeding stock farm in the south of Brazil. The mycoplasma-free status had been confirmed by the Brazilian Ministry of Agriculture by repeated serological testing by serum plate agglutination (SPA) and haemagglutination inhibition (HI) tests at 6 to 8 week intervals throughout the flock life, plus at least one attempt to isolate MS from tracheal swabs. In addition we carried out MS and MG polymerase chain reaction (PCR) on tracheal swabs from birds of three ages (19, 33 and 66 weeks) and confirmed the negative status.
Tracheal swabs for PCR and culture and blood samples for RSA and HI tests were also collected at 67 and 69 weeks from the 10 birds that were moved to isolators when the flock was slaughtered. These birds were necropsied at 71 weeks and culture and PCR was attempted from trachea, airsacs and hock joints. Internal organs and hock synovial membranes were examined for histological lesions (Luna, 1968).

Minimal inhibitory concentrations (MICs) for ENR, NOR and OXT were determined in vitro (Hannan, 2000) with the type strain WVU1853 and with two MS isolates (443 and 563) from the previous batch of birds on the same brooding and rearing farm. PHO was not included as its mode of action is to inhibit peptidoglycan synthesis and therefore it has no activity against the cell wall-less mycoplasmas.

### Results

PCR indicated that the MS-free flock that was placed on the MS endemic site was positive by 9 weeks of age and 70% or more swabs were PCR positive up to 29 weeks (Table 1). After treatments had commenced these fell to 1 in 10 PCR positive swabs by 34 weeks, thereafter there were no further positive PCR reactions. SPA reactions were first seen at 29 weeks and persisted until 59 weeks. By the time the flock was slaughtered at 67 weeks there were no positive SPA reactors and the 10 hens that were retained, including the five that received live IBV vaccine, remained PCR and SPA negative. No positive HI reactions were recorded during the entire study nor were signs or lesions related to MS seen in either the flock prior to slaughter or in the 10 retained birds. No microscopic lesions of airsacculitis or synovitis were seen in the tissues taken from these 10 birds.

MIC breakpoints were 2 μg/ml for OXT, 0.125 μg/ml for NOR and 0.25 μg/ml for ENR while the

### Table 1. Timetable of events and laboratory results for the flock from 0 to 69 weeks of age

<table>
<thead>
<tr>
<th>Week</th>
<th>PCR (of 10)</th>
<th>Isolation (of 1)</th>
<th>RSA (of 20)</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>MS-free flock housed on MS endemic farm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td>22</td>
<td>Flock moved to a clean farm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>nd</td>
</tr>
<tr>
<td>26</td>
<td>E. coli peritonitis diagnosed; 35 mg/kg phosphomycin in feed for 5 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>10 mg/kg enrofloxacin in feed for 5 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>0b</td>
</tr>
<tr>
<td>32</td>
<td>600 ppm oxytetracycline started in feed (continuous)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>1</td>
<td>0</td>
<td>20</td>
<td>nd</td>
</tr>
<tr>
<td>39</td>
<td>10/ mg/kg enrofloxacin for 5 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>nd</td>
</tr>
<tr>
<td>52</td>
<td>10 mg/kg norfloxacin for 5 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>nd</td>
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<tr>
<td>59</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>nd</td>
</tr>
<tr>
<td>67</td>
<td>Oxytetracycline withdrawn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>Flock slaughtered; 10 hens moved to isolators; 5/10 given 10 × IBV</td>
<td>0</td>
<td>0b</td>
<td>0b</td>
</tr>
<tr>
<td></td>
<td>Remaining 10 birds necropsied; no lesions seen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a out of 20 birds.
b out of 10 birds.

### Sampling and laboratory tests

Ten tracheal swabs for PCR and 10 more for mycoplasma isolation were collected at 9, 15, 23, 29, 34, 47, 54 and 59 weeks of age together with 20 blood samples. PCRs were carried out on individual swabs by the method of Lauerman et al. (1993). Mycoplasma isolation was attempted for each swab in 3 ml of broth (Frey et al., 1968) supplemented with 0.1 g/l nicotinamide adenine dinucleotide and 0.1 g/l cysteine hydrochloride. Broths showing pH change or turbidity were plated onto agar (Frey broth with 0.75% agarose). All other broths were plated out weekly for three weeks. Mycoplasma isolates were identified by immunofluorescence and filter-cloned three times (Bradbury, 1996).

SPA tests were performed on the farm on fresh sera using commercially prepared antigen (Intervet B.V., Boxmeer, the Netherlands) and the HI test (Kleven and Mycoplasmosis, 1998) was carried out on the serum collected at 29 weeks.

### Housing and flock history

The birds in this study were hatched at the grandparent company’s hatchery and brooded and reared on a high biosecurity farm with two 32 000 bird all-in all-out quarantine units. Asymptomatic MS infection, usually detected by serology and occasionally confirmed by isolation from tracheal swabs, had occurred in every flock housed in recent years on the farm. At 22 weeks the birds were transferred to a farm that had been depopulated, cleaned and disinfected. They were housed in four houses and 12 000 of one house formed the population for the continued study. After 26 weeks the flock showed recurrent E. coli peritonitis and received several in-feed antibiotic treatments. These included phosphomycin (PHO; Indern do Brasil), enrofloxacin (ENR; Faith SA) and norfloxacin (NOR; Formil Quima LTDA) (Table 1).

From weeks 32 to 59 in-feed oxytetracycline hydrochloride (OXT) (Huaishu Pharmaceutical Corp.; 600 ppm) was also given continuously. At 67 weeks the flock was slaughtered but 10 hens were retained and housed in isolators in two groups of five. One group was vaccinated by eye-drop with infectious bronchitis vaccine (Broilerbron-H120; Schering-Plough Coopers) at 10 times the recommended dose in an attempt to exacerbate any MS infection. All 10 birds were necropsied at 69 weeks.

### Winter PCR (of 10) Isolation (of 10) RSA (of 20) HI test

0 67 Flock slaughtered; 10 hens moved to isolators; 5/10 given 10 × IBV 0 0 0b 0b

Remaining 10 birds necropsied; no lesions seen
breakpoints for the type strain were 2 μg/ml for OXT and ENR and 1 μg/ml for NOR. All these were considered to be in the sensitive range.

Discussion

It has been reported that MS strains can differ in virulence (Fiorentin et al., 1991; Lockaby et al., 1998; Lockaby et al., 1999a; Lockaby et al., 1999b) and Morrow et al. (1990) suggested that variations in virulence may account for differences in the severity of the disease seen in broilers. There were no signs or lesions related to MS evident in the flock under study and the MS strain endemic on the farm seems to be of low virulence for broiler breeders and their offspring. Thus the lack of invasiveness of the MS may have been important for the success of its apparent eradication by the antibiotic treatments. The continued success of the treatment was probably related to the fact that at 22 weeks the flock was transferred to a clean farm, thus reducing the chances for reinfection from external sources. Several flocks emanating from the same rearing farm and housed untreated on other farms remained MS-positive by SPA until slaughter.

MS was not isolated in this study, which is somewhat surprising although Fiorentin et al. (2002) reported that MS isolation is more likely between 27 and 28 weeks of age and our samples were collected at 23 weeks of age and then only after enrofloxacin treatment at 29 weeks. Persistent yeast contamination of the swabs may also have prejudiced isolation attempts.

PCR proved more sensitive than isolation for detecting MS and was therefore useful for confirming the eradication of MS. HI tests were of low sensitivity and were negative when used on PCR positive birds. Antibodies were detected by SPA for many weeks after the PCR tests were negative but even these declined by the end of the study. These results suggest that surveillance for MS infection in antibiotic-treated flocks should not be based solely on serology but should be augmented by PCR.

Insufficient samples were tested to confirm that MS was completely eradicated from the flock by antibiotic treatment and attention to biosecurity measures but all PCR reactions were negative by 47 weeks. The administration of a large dose of live IBV vaccine at 67 weeks did not provoke any recrudescence of MS but only five birds were challenged in this manner.

Long term reliance on intensive antibiotic medication, particularly with fluoroquinolones, is no longer generally acceptable within the poultry industry but the targeted use of antibiotics in an eradication programme such as the one described here may reduce the need to medicate much larger numbers of birds in the next generation.

References


RÉSUMÉ

Eradicación de *Mycoplasma synoviae* chez des reproducteurs de type chair soumis à des traitements antibiotiques intensifs pour contrôler *Escherichia coli*

Un troupeau de reproducteurs de type chair indemne de *Mycoplasma synoviae* (*MS*) a été mis en place en période poulette dans une ferme où MS était endémique. En neuf semaines, les résultats PCR ont révélé que le troupeau s’était infecté. À l’âge de 22 semaines, le troupeau a été
transférent dans une ferme où la bande précédente a été éliminée et les bâtiments nettoyés et désinfectés. Les oiseaux ont été soumis à trois traitements de fluoroquinolone du fait qu'ils avaient présenté une péritonite récurrente à *Escherichia coli* et à partir de l’âge de 32 semaines, ils ont reçu 600 ppm d’oxytétracycline hydrochloride en continu dans l’aliment. Les recherches de MS par PCR ont révélé une diminution des oiseaux positifs après l’âge de 34 semaines et MS a dû être éradiqué à en juger par les résultats constamment négatifs en PCR. Nous en concluons que des traitements antibiotiques intensifs accompagnés de mesures de bio-sécurité adéquates peuvent éliminer MS de reproducteurs de type chair infectés.

**ZUSAMMENFASSUNG**

**Eradikation von *Mycoplasma synoviae* bei Broilerelterntieren nach intensiver antibiotischer Therapie zur Bekämpfung von *Escherichia coli***


**RESUMEN**

**Erradicación de *Mycoplasma synoviae* en reproductores sujetos a un tratamiento antibiótico intensivo para controlar *Escherichia coli***

Un lote de reproductores libre de *Mycoplasma synoviae* (MS) fue alojado en una granja endémica de MS durante la cría y recría. Mediante la técnica de PCR se detectó que el lote se infectó hasta las nueve semanas. A las 22 semanas el lote fue transferido a una caseta limpia y desinfectada en una granja que había sido despoblada. Las aves fueron tratadas tres veces con fluoroquinolonas debido a peritonitis recurrentes por *Escherichia coli* y a partir de las 32 semanas de edad recibieron 600 ppm de hidrocloruro de oxitetraciclina continuamente en el pienso. La monitorización frente a MS mediante PCR demostró una disminución de las aves positivas a MS tras las 34 semanas de edad y podría ser que el MS hubiera sido erradicado a juzgar por los resultados negativos obtenidos mediante PCR de forma consistente. Se concluye que un tratamiento antibiótico intensivo junto con unas medidas de bioseguridad adecuadas puede eliminar el MS de reproductores infectados.