



Eradication of *Mycoplasma gallisepticum* and *M. synoviae* from a chicken flock by antimicrobial injections in eggs and chicks

Erradicação de *Mycoplasma gallisepticum* e *M. synoviae* de um plantel de galinhas pela injeção de antimicrobianos em ovos e pintos

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ABSTRACT

A chicken breeding flock of 3,464 hens, naturally infected with *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS), was subjected to a mycoplasma eradication scheme, based on antimicrobial treatment of eggs and their hatched day-old chicks. The egg injection sites utilized were the air cell and the small end (albumen), and the antibiotics used were tylosin at two different doses (3 mg or 5 mg per egg) and gentamicin (0.6 mg/egg). For the chicks treatment, a combination of spectinomycin and lincomycin, diluted in dextrose-vitamin complex solution, was employed. The differences in hatchability for the egg air cell-embryo (17.2%), egg small end (albumen)-3mg tylosin (72.4%) and egg small end (albumen)-5mg tylosin (42.1%) injection procedures were significantly different by Chi-square analysis ($p < 0.0001$). Injection of antimicrobials into the air cell resulted in the highest drop in hatchability, followed by tylosin dose of 5 mg plus 0.6 mg of gentamicin into the egg small end, compromising the further genetic use of their hatched chickens. The best performance on hatchability (72.4%) was obtained when preincubated eggs were injected into the albumen with a combination of 3 mg of tylosin plus 0.6 mg of gentamicin. The difference in hatchability from 75.0% to 70.0% obtained, respectively, between lines A and B was not statistically significant. The F1 progenies from the antibiotic treated eggs stayed free from MG and MS until they reached the age of 12 months, when they were eliminated and replaced by their respective F2 substitutes.

Key words: chicken, *Mycoplasma gallisepticum*, *M. synoviae*, egg and embryo treatment, antimicrobial injection.

RESUMO

Um plantel de galinhas reprodutoras com 3.464 fêmeas, infectadas naturalmente por *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS), foi submetido a um processo de erradicação de micoplasma, com base no tratamento antimicrobiano de ovos e de seus respectivos pintos de um-dia de idade. Os sítios de injeção utilizados foram a câmara de ar e a ponta fina (albumina), sendo que os antimicrobianos usados foram tilosina em duas doses (3 mg ou 5 mg por ovo) e gentamicina (0,6mg/ovo). Para o tratamento dos pintos, uma combinação de espectinomicina e lincomicina, diluídos numa solução dextrose-complexo vitamínico, foi empregada. As diferenças em eclodibilidade para injeções na câmara de ar de cada ovo embrionado (17,2%), na ponta fina do ovo (albumina)-3mg tilosina (72,4%) e na ponta fina do ovo (albumina)-5mg tilosina (42,1%) foram significativamente diferentes pelo teste de Qui-quadrado ($p < 0.0001$). A injeção de antimicrobianos na câmara de ar resultou na mais elevada queda de eclosão, seguida pela dose de 5,0 mg de tilosina mais 0,6 mg de gentamicina na ponta fina do ovo, comprometendo utilizações genéticas futuras das galinhas nascidas desses ovos. O melhor desempenho em eclodibilidade (72,4%) foi obtido quando ovos pre-incubados foram injetados na ponta fina com uma combinação de 3,0 mg de tilosina mais 0,6 mg de gentamicina. A diferença em eclodibilidade de 75,0% para 70,0% obtida, respectivamente, entre as linhas A e B não foi estatisticamente significante. As progênes F1 dos ovos tratados com antimicrobianos permaneceram livres de MG e MS até a idade de 12 meses, quando foram eliminadas e substituídas por suas respectivas progênes F2.

Descritores: galinha, *Mycoplasma gallisepticum*, *M. synoviae*, egg and embryo treatment, antimicrobianos.

INTRODUCTION

Mycoplasma gallisepticum (MG), responsible for chronic respiratory disease (CRD), and *M. synoviae* (MS), that causes synovitis, are also the main cause of airsacculitis in poultry [6,13]. MG and MS infections can be transmitted horizontally (bird to bird), and vertically (through the egg), hence, affecting the breeding flocks [10]. The economic losses caused by mycoplasmosis are generally attributed to decreased laying performance, increased mortality and carcass condemnation rates, high medication cost, and synergistic effect associated with diseases or other factors [3,6]. Eradication of mycoplasma, based on its elimination into the egg, it is done without affecting the embryo development and subsequent hatchability of the mycoplasma-free progeny [3,5,6,9,14]. Informations on antimicrobial injection into the egg air cell, are found for chicken [10,11], but with respect to egg small end (albumen) it is found for turkey hatching eggs [1,5]. Published studies on injection of chicken eggs to eliminate mycoplasma are scarce, because this procedure is mostly conducted within the commercial poultry premises [14]. Tylosin is very efficient against mycoplasmas, while spectinomycin, lincomycin and gentamicin are broad-spectrum anti-microbials that work, preferably, against *Enterobacteriaceae* [8,9,15].

The objective of this study was to eradicate MG and MS from two chicken genetic stock lines, using procedures that included the injection of antibiotic into eggs, via small end and air cell, of MG and MS infected chicken, combined with antimicrobial treatment of day-old chicks, in order to provide an unbiased breeding selection, due to these mycoplasmas.

MATERIALS AND METHODS

Chicken flock informations

The chickens used were from a genetic stock of white-egg fowls (leghorn)¹. When the egg treatment began, there were 3,464 hens in the flock, which were being used for genetic improvement research, with age ranging from 45 to 53 weeks old (average of 48 weeks), and a laying production ranging from 37.0% to 48.0% (average of 42.0%). They were kept caged and artificially inseminated with semen from about 350 caged males. Out of these 3,462 hens, 1,929 constituted a sort of primary breeding flock (parent flock), which was divided into line "A" with

1,155 hens (father line) and line B with 774 (mother line). The others 1,535 hens were crosses between lines A and B, i.e., the multiplier breeding flock. These hens were housed in seven different groups, three of line A, two of line B, and two of A x B lines, of about 400 chickens, according to their exact ages. Prior to the beginning of this eradication scheme, all hens were tested for MG and MS infections by serum agglutination reaction (SAR) and hemagglutination inhibition (HI), as described [12]. Isolation of MG and MS in this flock was also accomplished [7,12], besides the history informations on the diagnosis of other disease [2].

Drugs and their doses used

For egg treatment, tylosin¹ and gentamicin sulfate² were diluted in 85.0% saline. A volume of 0.1 mL of saline solution with 3.0 mg or 5.0 mg of tylosin and 0.6 mg of gentamicin was inoculated per egg. Additionally, each day-old chick received a subcutaneous injection, containing 0.2 mL of a Linco-Spectin solution³ (5.0 mg of lincomycin and 10 mg of spectinomycin); 0.05 mL of 5.0% dextrose solution, and 0.05 mL of vitamin complex⁴.

Injection procedures

The eggs in trays were carefully inspected and dry-cleaned, whenever any dirt was seen on them. They were put air cell up or down, according to the chosen site of inoculation. The injection area of each egg, i.e., air cell or small end, were disinfected with iodine-alcohol solution applied gently with the help of a swab. Following disinfection, egg shell holes were made with a portable electric dentist drill⁵, attempting not to damage the shell membrane. The drills used were very small, and that made possible the absence of holes of 0.5-0.8 mm in diameter. An automatic syringe⁶ of 1.0 mL with needle of 4 mm x 5 gauges in size was used to deliver the antimicrobial solution into the drilled eggs. Between injections, the needle was scoured in iodine-alcohol soaked gauze, being the gauze exchanged whenever drying was taking place. Albumen expressed during injection was wiped from the injection site with the help of gauze soaked in alcohol. After injection, the eggs holes were sealed with melted paraffin wax.

The automatic syringe as described above with needle of 5 mm x 10 gauges in size was used to inject a solution of antimicrobial and dextrose-vitamin, sub-

cutaneously, in the dorsal region of the neck of each day-old chick.

Egg handling and treatment procedures

Eggs for each incubation/treatment batch were collected daily during a two-weeks interval. After each collection/day, the eggs were stored in a room with controlled temperature and humidity, where they received fumigation desinfection (formaldehyde gas) once or twice. Thereafter, the eggs were sent to the hatchery, located on the same premise, when they were fumigated again.

Eggs were treated and incubated in seven different batches, spaced about one week apart from each other. The numbers of eggs treated, the number of batches per line, the egg injection sites, and the combined doses of each antimicrobial used are presented under results (Table 1). The incubator and hatchery were cleaned thoroughly and disinfected before receiving the treated eggs. All eggs were fumigated at the entrance of the incubation room, after anti-microbial treatment, and when transferred to the hatchery. Incubated eggs in trays were removed from the incubation place, treated with antimicrobials, fumigated and immediately returned to their previous location. Eggs subjected to preincubation treatment, i.e., the albumen injected ones, were incubated about two hours after finishing the injection procedure. The

eggs were subjected to candling prior to incubation, and every four days from 4-17 post-incubation days, for the elimination of un-fertile eggs and/or dead embryos.

Management of the FI Progenies

Not all hatched chicks were used for subsequent breeding purpose. All saved chicks were subjected to sexing and most of the males were culled. The selected chicks were put in a chicken house located about 1,000 meters away from their mother hens, where they stayed until about 100 days, being, then, caged. By this time, their parents had already been culled, slaughtered and marketed a month before. The FI progenies were placed in houses and cages that had been cleaned, disinfected, and emptied for at least a month before use. Biosecurity measures, which included control of personnel, ration, vehicles, fomites, etc. were adopted.

Monitoring scheme

All pipped embryos from treated eggs were bled, sacrificed, necropsied and inspected for air sac lesions. All embryos with lesions, and only five without, from each incubation batch were subjected to culturing for mycoplasma isolation [7,12]. The sera obtained from the pipped embryos were pooled in groups of five and subjected to SAR and HI for MG and MS. SAR was

Table 1. Egg Parameters by Injection site and Treatment Procedure (0.6 mg of gentamicin plus tylosin at 3mg or 5mg).

Egg parameters	Egg treatment procedure			Total eggs used
	Air cell ¹ Tylosin 3mg	Albumen ² Tylosin 3mg	Albumen ³ Tylosin 5mg	
Layed	789	6,792	8,004	15,585
Fertiles	517	6,127	7,576	14,220
Hatched	89	4,436	3,194	7,719
Fertility	65.5%	90.2%	94.6%	91.2%
Pipped, # and (%) ⁴	7 (8.0%)	127 (2.9%)	180 (5.6%)	314 (4.0%)
Hatchability ⁵	17.2%	72.4%	42.1%	54.3%

1. One incubation with embryos, treated on the 8th incubation day, from line A hens.

2. Two incubations for line A eggs and two others for line B.

3. Two incubations with eggs from lines A x B hens.

4. Chi-square analysis, not significant (p=0.132)

5. Chi-square analysis, (P<0.0001).

performed with commercial antigens for MG⁷ and MS⁷ and the HI was conducted with laboratory made antigen⁹, with four hemagglutinating units, according to standard procedures [6,12].

The F1 progenies were monitored for MG and MS infections by serology (SAR and HI) and culturing. Culturing was performed on specimens from birds that appeared dead, sick, and from those apparently healthy, by the tracheal swab procedure [12]. Serology was performed when the chickens (100% of them) were aging 2-3 months, 5-6 months and 11-12 months (replacement time).

Statistical analysis

Chi Square test for heterogeneity with confidence interval of 95% [4] was used to compare the proportions of hatched, pipped and unhatched eggs from the three treatment groups under trial, as well as to investigate the genetic effect on hatchability, using eggs injected in the small end, and under the same antimicrobial dose (3 mg of tylosin plus 0.6 mg of gentamicin), from lines A and B.

RESULTS

Of the 3,464 hens tested prior to the beginning of the egg treatment, 3,118 (90.0%) and 2,078 (60.0%) were, respectively, positive for MG and MS by SAR and HI. These and other untyped mycoplas-

monellosis. Non-bacterial diseases had also been diagnosed as coccidiosis, aspergillosis, Marek's disease, Lymphoid leukosis, Newcastle disease, and Infectious Bursal Disease.

A total of 15,584 eggs with fertility rates ranging from 65.5% to 94.6% were subjected to antimicrobial treatment, and data pertaining to fertility, and hatching performance per treatment scheme (injection site and dose of tylosin) is being presented (Table 1). The hatchability for the air cell treated embryos (8-days of incubation) was drastically smaller (χ^2 analyzes, $p < 0.0001$) than that obtained for the small end, i.e., albumen site of preincubated eggs (Table 1). Increasing the tylosin dose from 3 mg to 5 mg reduced the hatchability from 72.4% to 42.1%, difference also statistically significant ($p < 0.0001$) by χ^2 analyzes (Table 1). The treatment scheme used, had no effect on pipped embryo frequency (Table 1).

The effect of the antimicrobial treatment, by the albumen route, on chickens from the lines A and B was investigated by χ^2 analyzes, but there was no statistical differences (table 2), although the hatchabilities obtained was 75.0% for line A, and 70.0% for line B (Table 2).

DISCUSSION

The antimicrobial egg treatment scheme described herein, which included the treatment of day-old chicks and biosecurity measures, was sufficient for the obtention of MG and MS-free chickens with only one application.

Tylosin was used because of its proven efficiency against mycoplasmas [8,15] and gen-

tamicin was used because of its broad-spectrum activity against bacteria and its low toxicity to host cells [15]. The use of gentamicin was justified also by the information on bacterial diseases, including non-pullorum salmonellosis, diagnosed in this genetic flock in previous years. Tylosin can be toxic for eggs when used in high doses as evidenced by the drop in hatchability, observed when the dose of this antibiotic was increased from 3 mg to 5 mg/egg.

The positive reactions obtained for sera from pipped embryos on the HI test can be explained by the

Table 2. Effect of egg treatment (3.0 mg of tylosin plus 0.6 mg of gentamicin) on two chicken lines¹

Line Incubated	Egg parameters, number and (%)				
	Fertiles	Hatched	Pipped	Fertility	Hatchability ²
A	3,457	2,998	2,247	66 (87.0)	(75.0)
B	3,515	3,129	2,189	61 (89.0)	(70.0)

1. Eggs injected into albumen.

2. Chi-square analysis, not significant ($p=0.429$).

mas had been isolated from necropsied birds from this flock several times in the past, prior to this eradication trial. The diagnosis of Chronic Respiratory Disease (CRD) and/or Airsacculitis with and without the possible involvement of *Escherichia coli* was also obtained in necropsied chickens, culled prior to this antimicrobial treatment trial.

As to the records of other diseases that were diagnosed in previous years, informations were available on other bacterial diseases, such as Infectious Coryza (*Haemophilus parallinarum*) and non-pullorum sal-

presence of maternal antibodies transmitted from hens to their eggs. The use of uninoculated eggs to serve as a control group was not employed due to the risk of compromising the eradication scheme adopted. The variation in fertility rates observed on different incubation batches of eggs may be explained by the way these hens were fertilized, i.e., by artificial insemination.

The better hatchability results obtained for eggs treated in the small end (albumen) over the air cell route has also been observed in the case of turkey eggs [5]. Because of the drastic mortality of embryos treated by the air cell route, this procedure was discontinued and the chicks hatched were not saved for breeding purposes. Besides, chicks hatched from eggs treated with 5 mg of tylosin, due also to high mortality, were not saved for breeding purposes, due to the fear of losing genetic variability, hence, compromising future crossings.

The antimicrobial treatment of eggs alone should not be judged responsible for the success of this eradication scheme, as day-old chick antibiotic treatment, biosecurity, and flock monitoring were also adopted. With the eradication of MG and MS, the genetic selection and breeding improvement can be carried out in this chicken genetic stock without the nega-

tive effects caused by the presence of MG and MS infections.

CONCLUSION

Antimicrobial treatment of preincubated eggs at the small end (albumen), in conjunction with treatment day-old chicks hatched from treated eggs, was sufficient to eliminate MG and MS from a genetic chicken stock flock with only one application.

SOURCES AND MANUFACTURERS

- ¹Experimental Station of Rio de Janeiro State Research Agricultural Organization-PESAGRO/RIO, Itaguaí, RJ, Brazil.
- ²Tylosin – “Tylan Soluvel, Elanco Química LTDA/Eli Lilly do Brasil LTDA, São Paulo, SP, Brasil”.
- ³Gentamicin Sulfate – Gentocin, “Indústria Química e Farmacêutica Schering S/A, Rio de Janeiro, RJ, Brasil”.
- ⁴Linco-Spectin solution – TUCO, “Divisão de Upjohn Produtos Farmacêuticos LTDA, São Paulo, SP, Brasil”.
- ⁵Vitamin Complex – “Poli Vitaminico SM-Forte, Laboratório Santa Marina”, Brasil”.
- ⁶Electric dentist drill – Dentalwerk Burmoos, GES. M.B.H., Salzburg, Austria.
- ⁷Automatic syringe – FHK PISTOR 1-FJ-96, Fujihira Industry Co. Inc., Japan.
- ⁸Commercial antigens for MG and MS – BIOVET S/A, Vargem Grande Paulista, SP, Brazil.
- ⁹Projeto Saúde Animal (PSA)/Embrapa Agrobiologia, Seropédica, RJ, Brazil.

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