Occurrence of *Mycoplasma synoviae* on commercial poultry farms of Pernambuco, Brazil

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The state of Pernambuco is the largest producer of eggs in the North and Northeast of Brazil and second one in the broiler production. Mycoplasmas are important avian pathogens, which cause respiratory and joint diseases that result in large economic losses. The aim of the present study was to investigate the occurrence of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in broilers and commercial laying hens in the state of Pernambuco, Brazil. Tracheal fragments were analyzed from 55 healthy broilers, 35 broilers with respiratory signs and 30 commercial laying hens with respiratory signs, from 24 commercial poultry farms, each sample was composed of a pool of five birds. The bacteriological exam, PCR and nested PCR were used for the detection of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS). All samples were negative in bacteriological isolation. In the PCR analyses, seven samples from birds with respiratory signs were positive for MS and one was positive for MG, the latter of which was confirmed as the MG-F vaccine strain. The occurrence of MS in chickens with respiratory signs may indicate inadequate sanitary management on poultry farms, favoring the propagation of mycoplasmosis.

**INDEX TERMS:** Mycoplasmosis, *Mycoplasma synoviae*, PCR, broilers, commercial laying hens, diagnosis.
INTRODUCTION

The intensification of production in the poultry industry leads to particular conditions that favor the occurrence and dissemination of infectious diseases, especially those that affect the respiratory tract (Minharro et al. 2001).

Mycoplasmosis is considered the respiratory disease with the greatest impact on all segments of poultry farming. *Mycoplasma gallisepticum* (MG) causes chronic respiratory disease and accounts for economic impact related to feed conversion loss, lower egg production, embryonic mortality and the non-approval of the carcass for human consumption (Yoder 1984). *Mycoplasma synoviae* (MS) causes subclinical infection of the upper respiratory tract, characterized by a lack of clinical signs or only respiratory illness (Stipkovits & Kempf, 1996). *M. synoviae* can also cause airsacculitis in chickens (Rosaes 1991) and is often found in its asymptomatic form on poultry farms in Brazil (Fiorentin et al. 2003). Following infection by *Mycoplasma gallisepticum* or *Mycoplasma synoviae*, chickens become more susceptible to secondary infections by other viral or bacterial agents, such as *Escherichia coli* (Alencar et al. 1998, Ferreira & Knöbl, 2000).

The aim of the present study was to investigate the occurrence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* on commercial poultry farms in the state of Pernambuco, Brazil.

MATERIALS AND METHODS

**Sampling.** Eleven farms with healthy broilers chickens, seven with broiler chickens presenting clinical respiratory signs and six farms with commercial laying chickens presenting clinical signs of respiratory disease were used, corresponding to approximately 20% of the poultry farms in the state of Pernambuco (northeastern Brazil). A total of 55 trachea fragments were collected from the healthy broilers, 35 were collected from broilers with clinical signs and 30 were collected from commercial laying chickens with clinical signs. Each sample was made up of a pool of five birds per farm, totaling 24 samples for the processing of the microbiological and molecular methods.

**Bacteriological exam.** After the collection of trachea samples by swabs, scarification and maceration, the specimens were diluted in series from 10^1 to 10^-5 and stored in glycerol (1:2) and frozen at -20°C until processing. For isolation, the samples were diluted in series from 10^1 to 10^-5, and then placed in a modified liquid and solid Frey medium and incubated at 37°C by 48-72h. The reactions in the agar were observed for 21 days under a stereomicroscope (40x) for the determination of the growth of characteristic colonies of *Mycoplasma* spp. (Nascimento 2000).

**DNA extraction.** DNA extraction of the swab, scarification and maceration samples was performed with the phenol/chloroform method (Sambrook et al. 1989).

**PCR protocols.** For the DNA amplification reaction, specific primers for *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS) and the MG-F vaccine strain were used (Table 1). Bacterial DNA was amplified in the PCR reaction mixtures as follows. Each reaction system had a final volume of 100µl and contained 59µl of ultra-pure water (Milli-Q), 10µl of 10X PCR buffer, 5µl of MgCl₂ (25mM), 5µl of dNTP mix (each 0.25mM), 2µl of each primer (100pmol), 2µl of Taq DNA polymerase (2.5 U/µl) and 15µl of the DNA template. The MG (ATCC 19610) and MS (ATCC 25204) strains from the American Type Culture Collection were used as the positive controls. The negative control consisted of the PCR water used in place of DNA. The mixtures were preheated at 94°C for 1 min before submitting to recycling step. The amplification conditions for the PCR assays were 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. A final extension step was performed at 72°C for 5 min and cooling at 4°C for 5 min.

**Nested-PCR.** The nested PCR was performed with the amplimers from the previous reactions using specific MG-PCR primers (Table 1). Each reaction system had a final volume of 100µl and contained 71µl of ultra-pure water (Milli-Q), 10µl of 10X PCR buffer, 5µl of MgCl₂ (25mM), 5µl of dNTP mix (0.25mM each), 2µl of each primer (100pmol), 2µl of Taq Polymerase (2.5 U/µl) and 3µl of the DNA template. The negative control consisted of the PCR water used in place of DNA. The mixtures were preheated at 94°C for 5 min before submitted to recycling step. The amplification conditions for the Nested-PCR assays were 40 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. A final extension step was performed at 72°C for 10 min and cooling at 4°C for 30 seconds. The PCR products were analyzed in agarose gel (1.5% w/v) and stained with ethidium bromide (5%) and the fragments were viewed under ultraviolet light. The 100-bp Ladder molecular weight marker was used.

**Statistical analysis.** The Kappa coefficient (K) was used for the concordance study, with the following conventional interpretation values: 0.00 to 0.20 = weak agreement; 0.21 to 0.40 = fair agreement; 0.41 to 0.60 = moderate agreement; 0.61 to 0.80 = good agreement; and 0.81 to 1.00 = very good agreement. Negative values were interpreted as equivalent to 0.0. The chi-square test of independence was used to determine associations between the biological materials (Landis & Koch 1977).

RESULTS

The clinical signs in the ill chickens were mild to abundant nasal drip, facial swelling and respiratory stertor. The necropsy findings were airsacculitis, hemorrhaging and muscular...
Mycoplasmas are pathogens that cause chronic respiratory disease, synovitis and bursitis in chickens and turkeys, accounting for considerable economic losses in the poultry industry. In Brazil, the frequency of infection by MS is greater than that by MG (Reis et al. 1973, Mettifogo et al. 2002). In the present study, the PCR results were positive in 38.46% of the samples that were positive for MS (Unpublished data). Another advantage of PCR is that infections combined with other mycoplasmas or other bacteria do not affect the reaction, making PCR a useful alternative for the diagnosis of avian mycoplasmosis (Nascimento et al. 1991). PCR is also chosen due to the fact that it is a specific, sensitive method capable of detecting and amplifying low-quality DNA (Saiki et al. 1985, Innis & Gelfand, 1990), which assists in farm monitoring programs and the differentiation of the field and vaccine strains of MG (Nascimento et al. 1993, Mettifogo et al. 2002). In the present study, the PCR results for MG differentiated the MG-F vaccine in samples from the commercial egg-laying farm with a history of vaccination.

CONCLUSIONS

Regardless of the type of poultry farm, Mycoplasma synoviae was the only species identified. Poultry producers in the state of Pernambuco, Brazil, should discuss control and biosafety measures for the prevention and propagation of this agent.

The use of PCR is indicated for the diagnosis and monitoring of mycoplasmosis on commercial poultry farms, is preferred to other methods, such as microscopy and culture, due to its high specificity and sensitivity. PCR is a useful alternative for the diagnosis of avian mycoplasmosis and other respiratory diseases in poultry.
also needed to differentiate MG field strain with the vaccine strain MG-F, included in the vaccination program in commercial laying farms.

**Research Ethics Committee.** The experimental protocol of this study followed the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation and received approval from the Ethics Committee on Animal Use of the University Federal Rural of Pernambuco (Brazil) under process no. 23082.001526.

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