Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Silva, CBC; Chagas, WF; Santos, RF; Gomes, LR; Ganda, MR; Lima, AMC
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Revista Brasileira de Ciência Avícola, vol. 17, núm. 1, enero-marzo, 2015, pp. 57-62
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Jan - Mar 2015 / v.17 / n.1 / 57-62
http://dx.doi.org/10.1590/1516-635x170157-62

Seroprevalence of Salmonella and Mycoplasma in Commercial Broilers, Backyard Chickens, and Spent Hens in the Region of Triângulo Mineiro, State of Minas Gerais, Brazil

■Author(s)

Silva CBC[I]
Chagas WF[I]
Santos RF[II]
Gomes LR[I]
Ganda MR[I]
Lima AMC[I]

[I] Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia.
[II] Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho , Campus Jaboticabal.

■Mail Address

Corresponding author e-mail address
Anna Monteiro Correia Lima
Av. Pará nº 1720, Campus Umuarama, Bloco 2T, Uberlândia, MG, CEP: 38400-902, Brazil.
E-mail: annalimaufu@yahoo.com.br

■Keywords

Salmonella pullorum, Salmonella gallinarum, Mycoplasma gallisepticum, Mycoplasma synoviae, Gallus gallus.

Submitted: February/2014
Approved: October/2014

ABSTRACT

Avian salmonellosis and mycoplasmosis are infectious diseases that, in addition of causing lack of flock uniformity, represent a hazard to human health. The objective of the present study was to evaluate the seroprevalence of mycoplasmosis and salmonellosis in commercial broilers, backyard chickens, and spent hens slaughtered at a processing plant with local health inspection in Uberlândia, MG, Brazil. A total of 210 samples were randomly collected at the time of bleeding. Samples were submitted to rapid plate serum agglutination test (RSA) for the classification of *Salmonella pullorum*, *Salmonella gallinarum, Mycoplasma gallisepticum* and *Mycoplasma synoviae*. In order to increase result specificity, mycoplasmosis-positive samples were submitted to hemagglutination inhibition test (HI). No samples presented detectable antibodies against *Salmonella pullorum* or *Salmonella gallinarum* in the RSA test. Only *Mycoplasma synoviae* was detected in 14% of the backyard chickens and 0.74% in commercial broilers, whereas no antibodies were detected in spent hens. The seroprevalence rates found in the present study emphasize the need of keeping chicken flocks free from disease using effective biosafety systems.

INTRODUCTION

The main infections that affect commercial poultry production are salmonellosis (Salmonella pullorum, Salmonella gallinarum, Salmonella typhimurium, Salmonella enteritidis) and mycoplasmosis (Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis), and are therefore included in the National Poultry Health Program (PNSA) (Brasil, 2010). These agents survive outside the bird's body, infecting both domestic and wild poultry by vertical and horizontal routes, and often do not cause clinical symptoms (Berchieri Jr., 2000). As a result, they are difficult to control, making biosecurity measures essential for the prevention of the infection of commercial flocks (Pereira, 2005).

Bacteria of the genus *Salmonella* cause avian salmonellosis and more than 2500 serotypes have been identified, among which 90 are frequently cause infections both in humans and in animals (Berchieri Jr. & Freitas Neto, 2009). Due to its wide distribution of among animals, the presence of carriers and their long survival in the environment and foods, *Salmonella* spp plays a relevant role in global public health, requiring permanent control and eradication programs (Cardoso & Tessari, 2008). Understanding the incidence and the prevalence of *Salmonella* serotypes in the different locations is essential for the establishment of preventive and control measures against the predominant serotypes (Hoffer *et al.*, 1997).

Salmonellosis in broilers includes three main diseases: pullorum disease, caused by *Salmonella pullorum*; fowl typhoid, caused by

Salmonella gallinarum; and avian paratyphoid disease, cause by serotypes related to food poisoning in humans. *Salmonella* Typhimurium and *Salmonella* Enteritidis are the most frequently agents isolated in food poisoning in humans, particularly in poultry products (Oliveira *et al.*, 2002).

Together with salmonellosis, mycoplasmosis is one of the main problems in the poultry production chain. Mycoplasma causes respiratory and joint diseases, resulting in significant economic losses (Buim *et al.*, 2005). The most important species in the poultry industry are *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), and *Mycoplasma meleagridis* (MM). These agents may cause endemic chronic diseases that are difficult to eradicate, and their presence in a farm result in slower growth rate, worse feed conversion ratio, increased mortality, lack of flock uniformity, reduced egg production, and increased downgrading in the processing plant.

The control of mycoplasmosis is critical in commercial poultry production because its eradication from flocks is very expensive. Therefore, the identification of serologically positive chickens is required for monitoring and, consequently, maintaining good health status of the flocks (Brasil, 2007).

Considering that Brazil is a global leader in poultry production, the importance of this activity for the country's economy, the impact of salmonellosis and of mycoplasmosis on productivity, and that these diseases represent significant health barriers for the exports of poultry products, the Brazilian Ministry of Agriculture (MAPA), through the National Poultry Health Program (PNSA), established by Ruling 193 as of September 19, 1994, developed programs for the control of salmonellosis and mycoplasmosis in Brazil (Brasil, 1994).

Considering the importance of the control and prevention of those diseases, the objective of the present study was to evaluate the seroprevalence of mycoplasmosis and salmonellosis in commercial broilers, backyard chickens, and spent hens slaughtered at a processing plant with local health inspection in Uberlândia, MG, Brazil.

MATERIALS AND METHODS

Sampling

Because there are no records on the prevalence of avian mycoplasmosis and salmonellosis for the studied chicken categories in the region of Uberlândia, and as determined by Normative Instruction 44 of MAPA, the number of samples was calculated according to an average prevalence of 10%, applying 90% confidence level and maximal estimate error of 4%.

As recommended by Vieira (2008), the following formula to determine the number of samples was:

$$\alpha = \frac{\left(\frac{z\alpha}{2}\right)^2 . \hat{p}.\hat{q}}{e^2}$$

$\frac{z\alpha}{2}$ = value of the normal distribution, which is 1.96 for a proportion of 90%

$\hat{p}$ = disease prevalence proportion

$\hat{q}$ = non-prevalence of the disease

e^2 = estimate error

The processing plant slaughtered 8,500 chickens monthly. This corresponded to an average of 2,060 backyard chickens, 5,445 commercial broilers, and 995 broiler breeders at the end of their production cycle, which are herein designated as spent hens. Applying the above equation, sample size was calculated as 210 chickens, out of which 50 were from backyard chickens, 135 from commercial broilers, and 25 from spent hens.

Blood samples

The experiment was conducted using non-probabilistic sampling, where samples were collected from all birds to be slaughtered coming from different farms. Blood samples were collected in a total of three collections at weekly intervals in a processing plant with health inspection of the local government of the city of Uberlândia, state of Minas Gerais, Brazil. Samples were collected during bleeding in sterile silicon plastic tubes, which were sealed after each collection. The blood tubes were duly identified, grouped according to bird classification, and submitted to the laboratory in isothermal containers.

Laboratory analyses

Samples were processed at the laboratory Laboratório Avícola Uberlândia Ltda. (LAUDO), which is accredited by MAPA to perform official mycoplasmosis and salmonellosis tests.

Rapid plate serum agglutination technique for fowl typhoid/pullorum disease, recommended by Ruling n. 126 as of November 3, 1995 (Brasil, 1995), was applied for the detection of *S. pullorum* and *S. gallinarum* antibodies.

Mycoplasmosis screening was performed using rapid plate serum agglutination test, and the positive

samples were submitted to hemagglutination inhibition test to enhance the specificity of the results.

Rapid plate serum agglutination for fowl typhoid/pullorum disease

Serum and antigen samples were at room temperature at the time of the analyses. An aliquot of 0.05mL of each sample was placed at the center of each plate square and to each sample, and a drop of positive-control serum was places in the last square of the plate. Then, 0.05mL of the rapid serum agglutination antigen for fowl typhoid/pullorum disease, prepared at the lab and previously homogenized, was added. Sample and antigen were agitated for two minutes by plate rotational movements, and then read. Samples were considered negative when no antigen-antibody agglutination was observed, that is, there were no changes in the mixture at the end of the two minutes of agitation. Samples were considered positive when the serum antigen mixture presented clots, indicating the formation of antigen-antibody complexes.

Rapid plate serum agglutination for the detection of antibodies against MG and MS

The rapid plate serum agglutination test (RSA) was performed according to the recommendations of Stanley & Yoder Jr. (1989) and of the antigen manufacturer (Laboratório Intervet do Brasil Ltda.) using Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) antigens. Serum samples and antigens were at room temperature at the time of the analyses.

Two equal aliquots of antigen and serum were added to the plate, homogenized, and after two minutes of mixing, the presence or absence of clots was observed. The test was carried out in standard glass plates, to which 50 µL of MG and MS antigens and 50 µL of serum were added. Sample and antigen were mixed with the aid of a multiple device. The serum-antigen mixture was agitated for two minutes by plate rotational movements with resting intervals.

The RSA test was performed with raw serum. Serum samples that were positive in the RSA test were diluted at 1:8 in 0.85% NaCl saline solution.

In order to increase the precision of classification of serum samples by RSA, only sera presenting agglutination at 1:8 dilution were considered positive for MG and MS. At this dilution, the serum-antigen mixture presented stained clots with different sizes, depending on the intensity of the reaction. These clots consist of the antigens (Mycoplasma gallisepticum and Mycoplasma synoviae) agglutinated by the antibodies present in the serum.

Samples were considered negative when there was no antigen-antibody agglutination, that is, the antigen-antibody mixture remained unchanged after two minutes of agitation.

Hemagglutination inhibition for the detection of antibodies against MG and MS

Samples detected as positive in the SAR test were tested for the presence of hemagglutination-inhibition (HI) antibodies. Hemagglutination inhibition tests are more specific than rapid plate serum agglutination tests and are used to confirm or not the serological response against MG and MS (Mendonça *et al.*, 2003).

Antigens containing four hemagglutination units (HAU) were used according to the technique standardized by Beard (1989). Titers were expressed by multiplying the number of hemagglutination units used by the reciprocal of the highest dilution where hemagglutination was completely inhibited.

RESULTS

Considering an average prevalence of 10%, with 90% confidence interval and 4% error estimate, it was observed that 100% of the sampled individuals did not present detectable antibody titer against *Salmonella pullorum* or *Salmonella gallinarum* in the RSA test.

Using the RSA test, nine (18%) backyard chickens were positive for *Mycoplasma gallisepticum* and 13 (26%) for *Mycoplasma synoviae*, totaling 22 (44%). There were two (1.48%) commercial broilers positive for Mycoplasma *synoviae*, whereas all spent hens were negative for the test *Mycoplasma* species. The RSA positive samples were submitted to the HI test, out of which seven (14%) backyard chickens and one (0.74%) commercial broiler were positive for *Mycoplasma synoviae* (Table 1).

DISCUSSION

The present study showed that none of the backyard chickens presented detectable antibodies against *Salmonella pullorum* and *Salmonella gallinarum* at the RSA test. Because backyard chickens are reared on farms with no biosecurity measures, it was expected that some birds would be positive.

Although the rearing conditions at the farms of origin were not known, we may speculate that these chickens were housed in semi-open or closed houses, which, despite possibly primitive, reduced the possibility of contact with free-living birds, other animals and visitor that may be contaminated.

Table 1 – Comparison between *M. gallisepticum*- and *M. synoviae*-positive chickens per category as detected by rapid plate serum agglutination test and by hemagglutination inhibition test.

		M. gallisepticum				M. synoviae			
Chicken class		RSA		HI		RSA		HI	
		+	%	+	%	+	%	+	%
Backyard chickens	50	9	18	0	-	13	26	7	14
Commercial broilers	135	0	-	-	-	2	1.48	1	0.74
Spent hens	25	0	-	-	-	0	-	-	-
Total	210	9	18	0	-	15	27.48	8	14.74

These results are consistent with Lima (2005), who found that 100% of blood samples collected from backyard chickens and tested by RSA were negative, possibly because these chickens were reared under relatively good biosecurity conditions.

On the other hand, Buchala *et al.* (2006) found a 16.5% seroprevalence of pullorum disease in backyard chickens, indicating circulation of this bacterium among these birds.

Also differently from the present study, Pereira (2005) in a study in Uberlândia, MG, Brazil, found 35.6% seropositive sera from 160 backyard chickens reared in farms neighboring large broiler breeders farms.

In the present study, none of the 135 serum samples collected from commercial broilers at market age were positive at the RSA for *S. pullorum* and *S. gallisepticum*. According to the PNSA, it is mandatory that these birds derive from breeders free from those bacteria. In addition, these broilers were theoretically reared in farms with high use of technology and good biosecurity conditions.

Consistent results were obtained by Lima (2005), who did not find any positive serum samples collected from market-age commercial broilers, possibly because they were submitted to adequate health management.

Out of the 25 serum samples collected from spent broiler breeders, reared in commercial farms, none were positive at the RSA test. This result shows compliance with the current PNSA regulation, which establishes that broiler breeders should be free from *S. pullorum* and *S. gallinarum*. Critical factors in broiler breeder farms, such as preventing flock contamination by infectious agents by biosecurity measures and permanent surveillance by laboratory test, are essential to eradicate *Salmonella* spp in such populations.

In an experiment carried out in 2003-2004, Tessari *et al.* (2005) collected 131,650 blood samples from broiler breeders, and detected 1,201 (0.91%) positive samples using RSA, out of which 259 (0.20%) were positive in the slow serum agglutination test, demonstrating low prevalence of the disease. Those authors also concluded that RSA is a simple test that can be routinely used for diagnosis and it is very sensitive to detect antibodies, despite not having good specificity. Shivaprasad (2003) reported that *Salmonella* spp is susceptible to adverse environmental conditions, and may destroyed in minutes if heated by the sun. It is possible that the environment conditions in which the birds were reared may have influenced the results of the present study.

Relative to mycoplasmosis, Buchala *et al.* (2006) tested 15 free range chicken farms and found seroprevalence of 73% for MG and 100% for MS. in the present study, a seroprevalence of 3.80% of backyard chickens was obtained for *Mycoplasma synoviae* using HI.

Pereira (2005), in a study on the prevalence of antibodies against MG and MS in 160 backyard chickens in Uberlândia, found that 102 (63.8%) were positive for MG using the RSA test and only 18 (11.3%) were positive at the HI test, whereas 102 (63.8%) were also positive for MS at the RSA test and 59 (36.9%) were positive at the HI test.

Bird management may explain the prevalence differences among chicken classes found in the present study (18% backyard chickens were positive for MG and 26% for MS in the RSA test, whereas only 14% were positive for MS in the HI test; and no commercial broilers were positive for MG and 1.48% were positive for MS in the RSA test, whereas only 0.74% were positive for MS in the HI test). This indicates that birds reared in farms where biosecurity programs are implemented are less contaminated than those reared in environments with no biosecurity measures.

However, the obtained percentage of Salmonella-positive backyard chickens was lower than that reported by Pereira (2005), possibly also to management factors. According to epidemiology concepts (Thrusfield, 2004), disease determinants may influence the frequency of

this disease in a population. Backyard chickens are not submitted to any standardized management, genetics, vaccination, or medications procedures, and, according to Walker (2003), age, environmental conditions, genetic predisposition, crowding, and concurrent infections may contribute to disease resistance.

In commercial broilers, the seroprevalence of *Mycoplasma synoviae* (1/210 or 0.46%) was low; however, this demonstrates that these birds had contact with this agent. Consistent results were obtained by Sentíes-Cué *et al.* (2005), who evaluated the frequency and severity of gross lesions associated with the infection of broilers by *Mycoplasma synoviae* in the state of California, USA, and found 15% positive birds using hemagglutination inhibition tests.

When HI, which has higher specificity, was used, only the presence of *Mycoplasma synoviae* was detected, suggesting cross reactions between *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in the RSA test. This may especially be the case of backyard chickens, as in general these birds are reared under poor health conditions and are exposed to several bacterial agents that may cause cross-reactions in serological tests.

Spent hens were 100% negative at the RSA test, differently from the findings of most studies in literature. Islam *et al.* (2014) testes layers and found 53.61% MG seroprevalence using the RSA test in Bangladesh. The findings of Cardoso *et al.* (2006) were consistent with the present study, with 100% of the tested broiler breeders negative for MG and only 1.58% positive for MS at the RSA test.

On the other hand, Mendonça *et al.* (2003) observed higher prevalence of MG (41.65%) compared with MS (11.10%) in a layer flock using the RSA test. Buim *et al.* (2005), tested commercial layers presenting respiratory problems with PCR, and found seroprevalences of 47.03% and 1.9% for MS and MG, respectively.

The dissemination of mycoplasmosis is commonly prevented by housing flocks of a single age in farms and by the application of adequate biosecurity measures, using the all-in, all-out systems. This system has been applied in many countries, making most of their broiler, turkey, and layer flocks free from this infection. Nascimento (2000) mentions that have flocks with multiple ages in a single farm allows the emergence of diseases and the dissemination of pathogens among birds of a same flock and among flocks.

Serological tests used for diagnosis when establishing health programs for the control or eradication of animal diseases because they allow processing a large number of samples. However, it should be considered that serological diagnosis may yield false positive or false negative results. Mendonça *et al.* (2004) attribute these errors to the low specificity of the RSA test, and consequently making this test not very reliable for the diagnosis of mycoplasmosis, particularly when it is the only test employed.

In order solve this issue, the National Poultry Health Program recommends that the tests should be regularly repeated so that the farm status as free from *Mycoplasma gallisepticum* e *Mycoplasma synoviae* for chickens and *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma melleagridis* for turkeys can be certified, according to Normative Instruction n. 44 of August 23, 2001.

The adaptation of the studied populations to their environment and to the evaluated agents is demonstrated by the absence of detectable antibodies in the SAR test against *S. pullorum* and *S. gallinarum* and the seroprevalence only of *Mycoplasma synoviae* in 14% of the backyard chickens, 0.74% in commercial broilers, and 0% in spent hens.

Attention to the need of surveying the presence of salmonellosis and mycoplasmosis in all poultry farms, independently of their technological levels in order to establish the prevalence of these diseases in many Brazilian regions, particularly in the state of Minas Gerais, due to its significant and widely diversified poultry production. While backyard chickens may be reared under reasonable biosecurity conditions, specific regulations for this type of production should be established by local, state, or federal authorities in Brazil because backyard chickens are still a significant mean of income in the country.

The seroprevalence rates found in the present study emphasize the need of keeping chicken flocks free from disease using effective biosafety systems.

ACKNOWLEDGEMENTS

The authors thank Laboratório Avícola de Uberlândia Ltda. (LAUDO) for its support with the analyses.

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