Seroprevalence of *Salmonella* and *Mycoplasma* infection in backyard chickens in the state of Entre Ríos in Argentina

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ABSTRACT The present work was conducted to study the seroprevalence of *Salmonella*, *Mycoplasma gallisepticum* (MG), and *Mycoplasma synoviae* (MS) infection in backyard chickens located in Entre Ríos, Argentina, over 3 periods of time. A total of 2,441 sera samples were collected from backyard chickens belonging to 256 family farms in 16 counties in the state of Entre Ríos from January to May 2003 (first period), December 2004 to April 2005 (second period), and October 2006 to May 2007 (third period). The prevalence of family farms testing seropositive for *Salmonella* averaged 23.9, 15.9, and 28.6% during the first, second, and third period, respectively. The highest prevalence of *Salmonella*-seropositive farms recorded (66.7%) was on farms from Concordia county, and the lowest prevalence (0%) was on farms from La Paz county. In contrast, the prevalence of family farms seropositive for MG averaged 32.8, 55.1, and 76.2% during the first, second, and third periods, respectively. The highest prevalence of MG-seropositive farms (100%) was found in the counties of Victoria and Tala, and the lowest prevalence (8.7%) was found on farms on Colón county. The prevalence of family farms seropositive for MS averaged 68.6 and 100% during the first and second periods, respectively. The highest prevalence of MS-seropositive farms (100%) was on farms in 85% of the counties tested, and the lowest prevalence (21.7%) was on farms from Colón county. *Salmonella*, MG, and MS infection are present at high levels in backyard chicken farms, and this presents a high risk to commercial poultry production in Entre Ríos, the state with the highest chicken population and density in Argentina.

Key words: *Salmonella*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, backyard chicken, Argentina

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INTRODUCTION

In Argentina, as in other developing countries, there is a sizable backyard poultry industry that, together with large-scale commercial producers, supplies domestic markets. The governments have adopted a system to breed backyard chickens and give them to low-income people so that they can have high-quality protein in their diets (Bonino and Canet, 1999). Local chicken production is a strategy for household poultry development in some countries, and backyard poultry farming is promoted because it can help the state to bridge the gap between the demand and supply of eggs and poultry meat, and can generate self-employment to reduce poverty and empower rural women. Furthermore, backyard or free-range evokes a positive image of chickens living outdoors with plenty of fresh air, sunshine, and open space to roam. These chickens may be purebred or hybrid, and they are not fed balanced feeds (Freire et al., 2005; Kperegbeyi et al., 2009; South Asia Pro Poor Livestock Policy Programme, 2009).

The insufficient farm management methods frequently applied in backyard chickens make them a possible reservoir for diseases such *Salmonella* and *Mycoplasma*, which can influence commercial poultry operations (Kelly et al., 1994). Infected poultry flocks are also among the most frequently implicated reservoirs of salmonellae that can be transmitted through the food chain to humans. Poultry producers are faced with intensifying pressure from public health authorities, elected officials, and consumers regarding food safety issues (Gast, 2003).

Infections of poultry with salmonellae can be grouped into 2 categories. One is infections with the 2 nonmotile serotypes, *Salmonella pullorum* and *Salmonella gallinarum*, which are generally host specific for avian species. The other is infections with the numerous motile

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Salmonella serotypes referred to collectively as paratyphoid salmonellae. Found almost ubiquitously in wild and domestic animals, this diverse group of serotypes is principally of concern as a cause of food-borne disease in humans (Gast, 2003; Shivaprasad, 2003).

Mycoplasmas (or mollicultes) are eubacteria devoid of cell walls and are the smallest self-replicating (able to be grown on artificial cell-free media) prokaryotes. *Mycoplasma gallisepticum* (**MG**) is the most pathogenic and economically significant mycoplasma pathogen of poultry. *Mycoplasma gallisepticum* infections are commonly known as chronic respiratory disease in chickens and infectious sinusitis in turkeys (Ley, 2003). In contrast, *Mycoplasma synoviae* (**MS**) infection occurs most frequently as a subclinical upper respiratory infection. It may cause air sac lesions when it is combined with Newcastle disease, infectious bronchitis, or both (Kleven, 2003).

Considering that no studies have yet been conducted on backyard or free-range poultry in Argentina, identifying and reducing the numbers of *Salmonella*, MG, and MS and their proximity to reservoirs may be important for the control of these diseases. Although a *Salmonella* (*S. gallinarum*, *S. pullorum*, *Salmonella enterica* serovar Enteritidis, *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Heidelberg) and *Mycoplasma* (MG and MS) control plan is included in the National Poultry Health Plan in Argentina (National Agrifood Health and Quality Service, 2003), backyard chickens play a significant socioeconomic role in poor communities, and their flocks are not usually monitored for diseases or vaccinated. Entre Ríos is a state in Argentina where poultry production is so concentrated that, from an epidemiological point of view, it is similar to a very large multiage farm. This state is divided in 17 counties and has almost 2,490 poultry houses, which correspond to 45% of the poultry houses from Argentina. It also accounts for almost 47% of the poultry production and 20% of the egg production in Argentina (Schell et al., 2010). Therefore, the present work was conducted to study the seroprevalence of *Salmonella*, MG, and MS infection in backyard chickens located in Entre Ríos, Argentina, over 3 time periods.

MATERIALS AND METHODS

Birds Sampling and Study Locations

A total of 2,441 sera samples were collected from backyard chickens belonging to 256 family farms and 16 counties in Entre Ríos state (Figure 1) in 3 time periods (Table 1). The first period, from January to May 2003, included 67 backyard farms in 5 counties; the second period, from December 2004 to April 2005, included 107 backyard farms in 8 counties; and the third period, from October 2006 to May 2007, included 84 backyard farms in 7 counties.

Four to 20 blood samples were taken from each backyard chicken farm, depending on the number of birds and the possibility of collecting them in these places. This meant that at least 25% of the birds in a given flock were sampled, and flock sizes typically consisted of 4 to 80 chickens. The age of the birds was between 14 wk old and more than 2 yr old, and the owners reported that their backyard chickens had not received any vaccinations to control the infections studied.

Blood samples were collected aseptically from the wing vein of birds using 5-mL sterile disposable syringes and needles. The samples were then kept at room temperature. The clear sera samples were poured into sterile vials, which were labeled and transferred to the INTA Laboratory of Poultry Health (Concepcion del Uruguay, Entre Ríos) in ice chests for the detection of *Salmonella*, MG, and MS infection by a rapid serum plate agglutination (**SPA**) test. *Mycoplasma synoviae* infection was not tested in the third period of study because a problem was encountered in obtaining the commercial MS antigen from the manufacturer.

Detection of Salmonella, MG, and MS Infection by SPA Test

The SPA test was performed according to the procedure described in the National Poultry Health Plan in Argentina (National Agrifood Health and Quality Service, 2003), with crystal violet-stained *Salmonella* (Nobilis SP), MG (Nobilis MG), and MS (Nobilis MS) antigens. The antigens used in this study were purchased from Intervet International BV (Boxmeer, the Netherlands). All sera samples was heated at 56°C for 30 min and cooled at room temperature. For testing, 0.02 mL of 1 antigen and 0.02 mL of chicken sera were placed side by side with a micropipette on a plastic plate, illuminated from below. The antigen and sera were then mixed thoroughly by stirring with a small toothpick, followed by rocking. Results of the SPA test were read within 2 min. In positive cases, granules were formed slowly, indicating that sera samples contained antibodies against *Salmonella*, MG, or MS infection. In negative cases, granules were not formed within 2 min, indicating that antibodies against *Salmonella*, MG, or MS were absent from the sera samples. Sera that reacted within 2 min were diluted 5-fold (1:5) in a physiological sodium chloride solution and retested in the first and second periods studied. The results of the SPA test were recorded. A family farm was considered positive when 25% or more serum samples from the farm were positive to the SPA test.

RESULTS

The results for seroprevalence of *Salmonella*, MG, and MS infections in backyard chickens from the first period of study are presented in Table 2. The seropositive family farms averaged 23.9, 32.8, and 68.9% for *Salmonella*, MG, and MS, respectively. Colón was the county with the lowest rate of seropositive farms, and

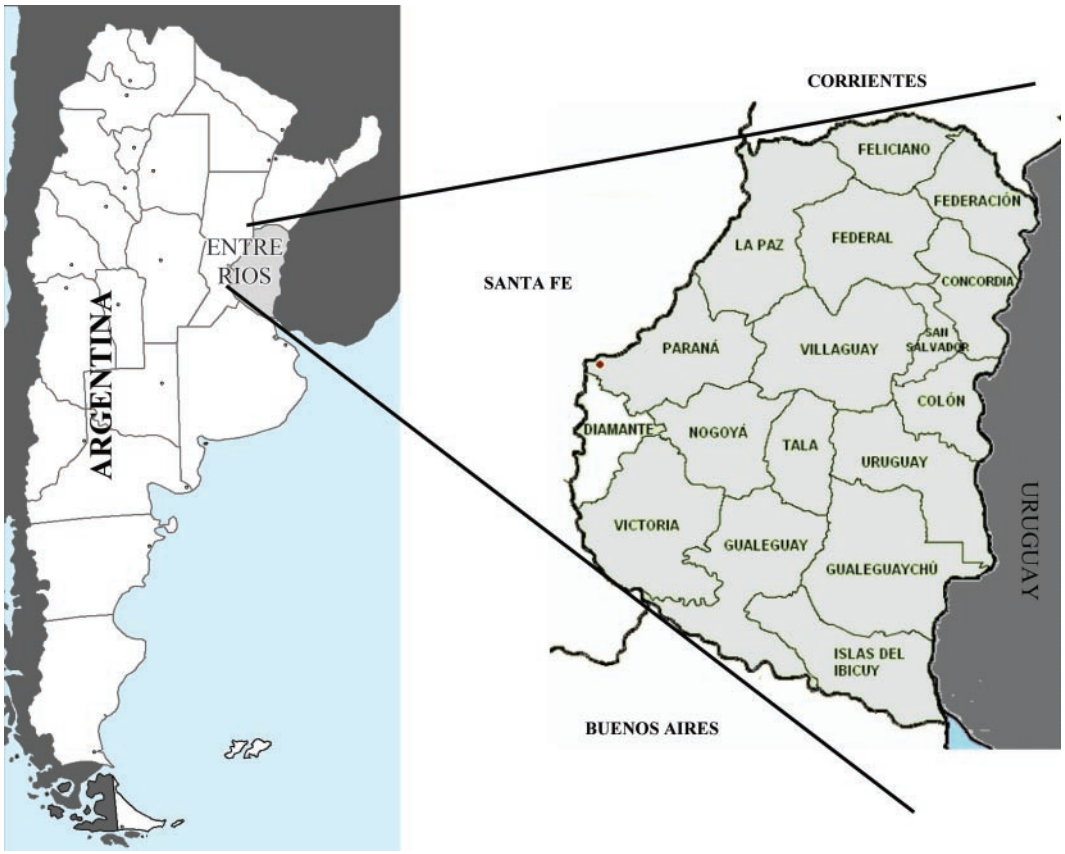

Figure 1. Regions with backyard chicken farms in Entre Ríos, Argentina, sampled for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Salmonella* seroprevalence studies over 3 periods from January 2003 to May 2007. The counties sampled are in light gray.

Concordia, Gualeguaychu, and Tala counties showed the highest rates of the diseases studied. *Salmonella* seropositive results were recorded for 8.7% (Colón county) to 66.7% (Concordia county) of farms. The family farms seropositive for MG were between 8.7% (Colón) and 66.7% (Gualeguaychu), and those seropositive for MS were between 21.7% (Colón) and 100% (Concordia, Gualeguaychu, Tala), respectively.

The family farms seropositive for *Salmonella*, MG, and MS averaged 15.9, 55.1, and 100%, respectively,

Table 1. Number of family farms and sera samples tested in the different counties from Entre Ríos during the 3 periods of study from January 2003 to May 2007

	First period (January to May 2003)		Second period (December 2004 to April 2005)		Third period (October 2006 to May 2007)	
County	No. of farms	No. of sera samples tested	No. of farms	No. of sera samples tested	No. of farms	No. of sera samples tested
Colón	23	186	—	—	28	260
Concordia	9	78	—	—	—	—
Federación	—	—	20	282	—	—
Federal	—	—	21	194	—	—
Feliciano	—	—	16	80	—	—
Gualeguay	—	—	—	—	12	180
Gualeguaychú	9	81	—	—	—	—
Islas del Ibicuy	—	—	8	61	—	—
La Paz	—	—	15	131	—	—
Nogoya	—	—	—	—	5	62
Paraná	—	—	—	—	7	64
San Salvador	—	—	13	115	—	—
Tala	5	47	—	—	8	110
Uruguay	21	185	—	—	17	174
Victoria	—	—	6	36	7	76
Villaguay	—	—	8	39	—	—
Total	67	577	107	938	84	926

Table 2. Prevalence of backyard chicken farms in counties from Entre Ríos seropositive for *Salmonella*, *Mycoplasma gallisepticum* (MG), and *Mycoplasma synoviae* (MS) from January to May 2003 (first period)

County	No. of farms	No. of farms seropositive for MG (%)	No. of farms seropositive for MS (%)	No. of farms seropositive for *Salmonella* (%)
Colón	23	2 (8.7)	5 (21.7)	2 (8.7)
Concordia	9	3 (33.3)	9 (100)	6 (66.7)
Gualeguaychú	9	6 (66.7)	9 (100)	2 (22.2)
Tala	5	3 (60.0)	5 (100)	2 (40.0)
Uruguay	21	8 (38.1)	18 (85.7)	4 (19.0)
Total	67	22 (32.8)	46 (68.6)	16 (23.9)

during the second period (Table 3). La Paz was the county with the lowest number of seropositive family farms, and the counties of Victoria and Federación showed the highest rate of seropositive family farms for the diseases studied. The family farms recorded as seropositive for *Salmonella* ranged from 0% (La Paz) to 40.0% (Federación). The family farms testing seropositive for MG ranged from 13.3% (La Paz) to 100% (Victoria), and 100% of farms from all the counties studied were seropositive for MS.

The overall percentages of farms seropositive for salmonellosis and mycoplasmosis (only MG) were 28.6 and 76.2%, respectively, in the third period of study (Table 4). The counties of Nogoya, Victoria, and Paraná had the lowest rates of seropositive farms for the diseases studied in backyard chickens, whereas Tala county showed the highest rate of seropositive farms. The farms seropositive for *Salmonella* in backyard chickens ranged from 14.2% (Victoria county) to 37.5% (Tala county), whereas the percentage of farms seropositive for MG ranged from 57.1% (Paraná county) to 100% (Tala county).

DISCUSSION

In the present study, an SPA test was performed to determine the seroprevalence of *Salmonella* and *Mycoplasma* (MG and MS) infection in backyard chickens in Entre Ríos, Argentina. Variation in the prevalence in different regions might be due to management problems (biosecurity, degree of infection in the litter, feed) and the rearing system.

Serological procedures are useful for flock monitoring in *Mycoplasma* and *Salmonella* control programs and to aid in diagnosis when infection is suspected (Ley, 2003; World Organization for Animal Health, 2008). The ELISA and hemagglutination inhibition test can also be used for detecting antibodies against *Salmonella* and *Mycoplasma* infection in chickens. Avakian et al. (1988) and Sikder et al. (2005) stated that the SPA test is very simple and sensitive, and can be used for the detection of both *Salmonella* and MG infection. The SPA test is highly efficient in detection IgM antibody, which is the first class of immunoglobulin produced in response to infection (Kleven, 1975).

The SPA test may sometimes show a false positive reaction. To overcome this problem, the test sera should be inactivated by heating at 56°C for 30 min in a water bath. Furthermore, certain nonspecific SPA reactions may be reduced by diluting the test serum (World Organization for Animal Health, 2008). These 2 processes were done in our assay.

It is well recognized that some animals or poultry with a positive serological response may no longer be infected with *Salmonella* organisms. Likewise, animals or poultry that are actively excreting salmonellae may be serologically negative. Animals or poultry that are serologically positive may have ceased to excrete salmonellae even though circulating immunoglobulin concentrations may remain high, whereas other animals or

Table 3. Prevalence of backyard chicken farms in counties from Entre Ríos seropositive for *Salmonella*, *Mycoplasma gallisepticum* (MG), and *Mycoplasma synoviae* (MS) from December 2004 to April 2005 (second period)

County	No. of farms	No. of farms seropositive for MG (%)	No. of farms seropositive for MS (%)	No. of farms seropositive for *Salmonella* (%)
Federación	20	16 (80.0)	20 (100)	8 (40.0)
Federal	21	8 (38.1)	21 (100)	2 (9.5)
Feliciano	16	12 (75.0)	16 (100)	1 (6.3)
Islas de Ibicuy	8	4 (50.0)	8 (100)	1 (12.5)
La Paz	15	2 (13.3)	15 (100)	0 (0.0)
San Salvador	13	7 (53.8)	13 (100)	2 (15.4)
Victoria	6	6 (100)	6 (100)	2 (33.3)
Villaguay	8	4 (50.0)	8 (100)	1 (12.5)
Total	107	59 (55.1)	107 (100)	17 (15.9)

Table 4. Prevalence of backyard chicken farms in counties from Entre Ríos seropositive for *Salmonella* and *Mycoplasma gallisepticum* (MG) from October 2006 to May 2007 (third period)

County	No. of farms	No. of farms seropositive for MG (%)	No. of farms seropositive for *Salmonella* (%)
Colón	28	21 (75)	8 (28.6)
Gualeguay	12	9 (75)	3 (25)
Nogoya	5	4 (60)	1 (20)
Parana	7	4 (57.1)	2 (28.6)
Tala	8	8 (100)	3 (37.5)
Uruguay	17	13 (76.5)	6 (35.3)
Victoria	7	5 (71.4)	1 (14.2)
Total	84	64 (76.2)	24 (28.6)

poultry on the farm may still be infected. Serologically negative animals or poultry may result from a recent infection, causing excretion before immunoglobulin production is maximal or infection with less invasive serotypes. Animals or poultry that have recently been infected would, in all probability, eventually be detected serologically by an appropriate monitoring program throughout the life of the flock or herd (World Organization for Animal Health, 2008).

Gast (1997) reported that positive culturing results for *S. pullorum* in the livers and ovaries of infected hens were almost always predicted by positive serological test results. The SPA test for *Salmonella* can detect *S. pullorum* or *S. gallinarum* (Shivaprasad, 2003), and it can underestimate the infection of the flock with other *Salmonella* serotypes. However, it is known that the coexistence of *S. gallinarum* and *Salmonella* Enteritidis in poultry prompts competition because of the shared immunodominant O9-antigen, which generates cross-immunity (Lee et al., 2001). Therefore, other invasive *Salmonella*, such as *Salmonella* Enteritidis, may give false-positive results in serological tests for *S. pullorum* (World Organization for Animal Health, 2008). Therefore, the SPA for *Salmonella* can also detect *Salmonella* Enteritidis infection. On the other hand, Terzolo et al. (1977) reported that 4 *S. pullorum* polyvalent commercial antigens reacted with sera containing somatic agglutinin 1 and with *Escherichia coli* B antiserum. Furthermore, Islam et al.(2006) found that the rate of *Salmonella* isolation from seropositive birds was much lower than that from seronegative birds.

Salmonella pullorum disease or fowl typhoid outbreaks in commercial layer flocks in connection with backyard flocks have been reported in Denmark (Christensen et al., 1994), Germany (Hinz et al., 1989), and the United States (Johnson et al., 1992). Pereira and Silva (2004a) found that 88.9% of Caipiras bird farms near commercial breeder flocks in Uberlandia, Brazil, were positive for *Salmonella* by the SPA test. On the other hand, Baruta and Ardoino (1999) tested 352 birds from different breeders for salmonellosis in the General Pico Agrarian Exhibition, La Pampa province, Argentina, and they found only 16 birds positive by SPA.

Some potential reservoirs of MG and MS carrier birds are backyard flocks (Ewing et al., 1996; Pereira and Silva, 2004b). Different studies have reported MG and MS infections on commercial poultry in La Pampa province (Baruta et al., 2001) and in Entre Ríos province (Cerdá et al., 1999), Argentina.

It is known that nonspecific reactors occur in some flocks when using the SPA test for MS, especially in flocks that have been vaccinated against various agents with oil emulsion vaccines. The MG antigen may be agglutinated on occasion, but the reaction is somewhat delayed and is usually lower in titer (Kleven, 2003; Feberwee et al., 2005). The backyard farmers in our studies did not vaccinate chickens against *Salmonella* and *Mycoplasma*. As a result, the chance of a false positive reaction was very small. On the other hand, a *Mycoplasma imitans* (**MIM**) infection can be confused with MG, or possibly with MS, because of serological cross-reactions (Bradbury et al., 1993; Feberwee et al., 2005). The MIM was originally identified as MG by immunofluorescence and growth inhibition tests, but subsequent serological and molecular studies indicated only a partial relationship to this species (Bradbury et al., 1993; Marois et al., 2001). At present, no evidence exists for the presence of MIM in commercial poultry because, to date, this bacterium has been isolated only from ducks, geese, and partridges (Bradbury et al., 1993) and it may have pathogenic potential in vivo (Abdul-Wahab et al., 1996).

The current seroprevalence study revealed that *Salmonella*, MG, and MS infection is widespread in backyard chicken farms, and this poses a high risk to commercial poultry production in Entre Ríos, the state with the highest chicken population and chicken density in Argentina. However, the losses caused by these bacteria were not estimated in the present study. It is suggested that backyard chickens be checked periodically to determine the status of *Salmonella* and *Mycoplasma* infection. The seropositive birds should be culled to take effective control measures against them. Isolation and identification of the etiological agent can be carried out to confirm the infection. On the other hand, because the backyard poultry system is being used to alleviate poverty through increased production, losses caused by *Salmonella* and *Mycoplasma* infection may impede this goal.

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