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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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# Differences of Seropositivity against Selected Infectious Agents in Relation to Farm Management Characteristics to Broiler Production in Uruguay

G. Rodríguez[1,2], K. Suzuki[1], M. Petruccelli[1], G. Trenchi[1,2], G. Giossa[1,2] and H. Trenchi[2]
[1]Laboratorio de Diagnóstico de Enfermedades de las Aves y los Pilíferos,
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina
[2]Área de Patología y Producción Avícola, Facultad de Veterinaria,
Universidad de la República, Montevideo, Uruguay

**Abstract:** The objective of this study was to investigate risk factors, especially for management characteristics associated with the seropositivity of respiratory infectious agents such as avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in broiler chickens on farms in Uruguay. Seventeen farms of broiler chickens (>35 days old) were studied between October 2008 and April 2009, comprised data collection through questionnaire interviews for each study farm, in combination with blood sample collections for each chicken (n = 1861). Of all the 17 study farms, 13, 13, five and nine farms were classified as seropositive against avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, respectively. The seropositivity against *Ornithobacterium rhinotracheale* in relation to the use of vaccination programmes indicated statistical significance. The farms with the vaccination programmes would be more likely to practise the same or similar sanitary measures, which could contribute to prevent the chickens from various infection. Practice of a sanitary measure would be indicative of a poultry farm to distinguish potential risks of diseases.

**Key words:** Poultry farming operations, questionnaire, South America

## INTRODUCTION

Respiratory infections in chickens are seen worldwide but especially in temperate poultry-producing areas in winter months. A number of respiratory viruses and bacteria may be involved. Avian pneumoviruses cause a severe respiratory infection in chickens. The virus has also been associated with swollen head syndrome in chickens (Cook, 2000). *Ornithobacterium rhinotracheale* infection is a transmissible disease of chickens that causes respiratory distress, mortality and decrease growth. The severity of clinical signs, duration of the disease and mortality are highly variable and influenced by environmental factors such as poor management (Van Empel *et al*., 2008). *Mycoplasma gallisepticum* is the etiological agent of chronic respiratory disease and causes reductions in egg production, hatchability and feed efficiency and increases in mortality and carcass condemnation (Ley, 2003). *Mycoplasma synoviae* may be responsible for infectious synovitis in chickens resulting in severe economical losses due to retarded growth and downgrading at slaughter. Most frequently, *Mycoplasma synoviae* infection results in a chronic subclinical upper respiratory infection (Kleven, 2005). In recent years, some field investigations regarding these infectious agents mentioned above in chickens were carried out in Uruguay (Giossa *et al*., 2010; Suzuki *et al*., 2010a; Suzuki *et al*., 2010b). These investigations were descriptive studies based on determining simply prevalence. No quantitative epidemiological investigations to identify risk factors for infection of the agents mentioned above in chickens in Uruguay have been publicized to the best of the authors' knowledge. The objective of this study was to investigate the risk factors, especially for management characteristics associated with the seropositivity of avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in chickens on broiler farms in Uruguay.

## MATERIALS AND METHODS

**Study area:** Uruguay is located in the south-eastern part of South America with a human population of 3.5 million. Uruguay has a poultry population of 14 million, a poultry meat production of 45,000 tones per year and a poultry egg production of 43,600 tones per year (FAO, 2009). The south of the country including the capital city Montevideo (34°53' S, 56°10' W) and Canelones Department has the concentration of chicken population (about 90% of the total), because of the largest national market Montevideo (Ministerio de Ganadería Agricultura y Pesca, 2009).

**Sample collection:** Seventeen farms of broiler chickens (>35 days old) were studied. Each study farm was

**Corresponding Author:** K. Suzuki, PROVETSUR, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina

selected from the capital city Montevideo, Canelones and Lavalleja (east of Canelones) Departments. None of the chickens had been inoculated against avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* prior to sampling. The required sample size of 1537 in total from a chicken population of 14 million was sufficient to obtain a 95% confidence interval (95% CI) with a desired precision of ±2.5% when the estimated seroprevalence was 50% (Hintze, 2008). The sample size in each of the farms was proportionally assigned (1% each of the total number of chickens at study farms) by available financial, human and material means. The field study was implemented between October 2008 and April 2009, comprised data collection through questionnaire interviews for each study farm, in combination with blood sample collections for each chicken. A questionnaire was designed to obtain basic information about management characteristics of the study farms. Major questions related to numbers of broilers (at the visit/shipped to market per year), vaccination programmes conducted, various sanitary measures as well as hatchery of origin.

**Laboratory examinations:** Blood samples were used for diagnostic tests. Individual-chicken sera were analyzed using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antibody against avian pneumovirus [FlockChek® (1) Avian Pneumovirus; (2) *Ornithobacterium rhinotracheale*; (3) *Mycoplasma gallisepticum* and (4) *Mycoplasma synoviae* Antibody Test Kit, Dr Bommeli AG, a subsidiary of IDEXX Laboratories, Liebefeld-Bern, Switzerland]. Positive and negative controls were included for each assay. Absorbance was read on an ELISA reader at 650 nm. Based on the instruction manual of the ELISA kits, serum samples with Sample to Positive (S/P) ratios greater than (1) 0.2 (titres larger than 396); (2) 0.4 (titres larger than 844); (3) 0.5 (titres larger than 1076) and (4) 0.5 (titres larger than 1076) were considered seropositive against avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, respectively. A farm was classified as positive against any infectious agents if at least one individual serum sample included in the farm was diagnosed as seropositive on the basis of the definitions above.

**Data analysis:** Data were entered into a database using the Base in the OpenOffice.org software version 3.1.1 (Sun Microsystems, Santa Clara, CA, USA). The statistical analyses were performed using Stata SE 10.1 (Stata Corporation, College Station, TX, USA). Univariate analyses using Fisher's exact test were conducted to describe the differences between seropositivities against avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and (1) the two farm groups classified according to practicing vaccination programmes and also (2) the other three farm groups classified according to hatcheries where the broiler chicks were introduced. Fisher's exact test were employed instead of Pearson's chi-squared test because the number of samples i.e. study farms was not large enough (Bland, 2000). After employing Fisher's exact test, risk ratio having seropositivity with statistical significance against an infectious agent was calculated (Dohoo *et al*., 2003).

## RESULTS

The 17 study farms had 187,400 broilers in total at the visit (equivalent to 1% of the total chicken population in Uruguay) and planned to have 1.7 million broilers shipped to market per year. Table 1 shows some management characteristics of the study farms. Eight farms responded that they have practiced the unique vaccination programmes against Marek's disease, infectious bronchitis and infectious bursal disease. Information about practicing vaccination programmes was not obtained from the other nine farms. Six and two farms introduced broiler chicks to the farms from the particular hatcheries A and B, respectively. Information about using any particular hatcheries was not obtained from the other nine farms. With respect to sanitary measures, all the study farms responded that (1) poultry houses were disinfected before placement using fumigation with appropriate chemicals such as formaldehyde; (2) home-type rodent control materials were used; (3) efficient fly control was conducted; (4) no animals were presented at the farm, other than chickens; (5) foot bath for disinfecting boots were used before entering poultry houses; (6) three persons as producer and one person as extensionist were permitted to enter poultry houses (7) feeders and drinkers were frequently maintained and (8) drinking water for chickens was from private source. Blood samples collected from 1861 chickens in the study farms were serologically investigated. Of all the 17 study farms, 13 [76%, 95% CI (50-93%)], 13 [76%, 95% CI (50-93%)], five [29%, 95% CI (10-56%)] and nine [53%, 95% CI (28-77%)] farms were classified as seropositive against avian pneumovirus, *Ornithobacterium rhinotracheale*, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, respectively. Table 2 describes the relationship between those seropositivities and use of vaccination programmes. Note that the vaccination programmes are against Marek's disease, infectious bronchitis and infectious bursal disease, not against the four infectious agents mentioned above. The seropositivity against *Ornithobacterium rhinotracheale* in relation to the use of vaccination programmes indicated statistical significance (Fisher's exact p =

Table 1: Selected management characteristics of the 17 study broiler farms in Uruguay

| Farm | No. of broilers at the visit | No. of broilers shipped to market per year | Vaccination programmes* | Hatchery† |
|---|---|---|---|---|
| 1 | 9 200 | 50 600 | n/a | n/a |
| 2 | 9 000 | 49 500 | + | n/a |
| 3 | 7 900 | 43 450 | n/a | A |
| 4 | 9 900 | 54 450 | + | n/a |
| 5 | 10 000 | 55 000 | + | n/a |
| 6 | 10 000 | 55 000 | + | n/a |
| 7 | 10 000 | 55 000 | n/a | B |
| 8 | 12 000 | 66 000 | n/a | A |
| 9 | 11 200 | 61 600 | + | n/a |
| 10 | 12 000 | 308 000 | n/a | A |
| 11 | 20 000 | 220 000 | n/a | A |
| 12 | 6 500 | 35 750 | + | n/a |
| 13 | 22 400 | 330 000 | n/a | A |
| 14 | 22 300 | 122 650 | n/a | A |
| 15 | 3 000 | 126 500 | + | n/a |
| 16 | 8 000 | 44 000 | + | n/a |
| 17 | 4 000 | 22 000 | n/a | B |

* +; practicing the same vaccination programmes against Marek's disease, infectious bronchitis and infectious bursal disease, † A and B; indicating two different hatcheries A and B, n/a; answers not available

Table 2: Number of the farms, with seropositivity against various infectious agents, practicing and non-practicing the vaccination programmes (n = 17)

| | Vaccination programmes classification* | | |
|---|---|---|---|
| Infectious agents | Practicing farms (n = 8) | Non-practicing farms (n = 9) | Fisher's exact P |
| Avian pneumovirus | 5 | 8 | 0.29 |
| *Ornithobacterium rhinotracheale* | 4 | 9 | 0.03 |
| *Mycoplasma gallisepticum* | 2 | 3 | 1.00 |
| *Mycoplasma synoviae* | 4 | 5 | 1.00 |

*The vaccination programmes are against Marek's disease, infectious bronchitis and infectious bursal disease, not against the infectious agents mentioned in the table

Table 3: Number of the farms, with seropositivity against various infectious agents, based on the hatcheries where the broiler chicks were introduced (n = 17)

| | Hatchery classification* | | | |
|---|---|---|---|---|
| Infectious agents | A (n = 6) | B (n = 2) | n/a (n = 9) | Fisher's exact P |
| Avian pneumovirus | 5 | 2 | 6 | 0.77 |
| *Ornithobacterium rhinotracheale* | 6 | 2 | 5 | 0.12 |
| *Mycoplasma gallisepticum* | 2 | 0 | 3 | 1.00 |
| *Mycoplasma synoviae* | 3 | 1 | 5 | 1.00 |

*A and B; indicating two different hatcheries A and B, n/a; answers not available

0.03). The risk ratio of the use of vaccination programmes having seropositivity against *Ornithobacterium rhinotracheale* was 0.3 (95% CI: 0.1-0.7). Table 3 explains the relationship between those seropositivities and hatcheries where the broiler chicks are introduced. The seropositivities against any infectious agents studied in relation to the hatcheries had no statistical significance (Fisher's exact p = 0.12-1.00).

## DISCUSSION

Nine study farms did not answer the question about practicing particular vaccination programmes. It does not mean that those farms do not adopt any vaccination programmes, and there is a possibility practicing the same vaccination programmes of the other five farms responded. No response from nine farms was obtained regarding the use of any particular hatcheries. It does not mean that those farms do not introduce chicks from the hatcheries A or B. All the farms responded that they have implemented eight good sanitary measures, particularly for maintaining poultry house hygiene. It is known that poultry farms in Uruguay must comply with strict national hygienic regulations as an indispensable prerequisite for poultry farm operations (Ministerio de Ganadería Agricultura y Pesca, 2010). Another potential source of these exemplary answers is that farmers may wish to present a positive image of themselves. There is a statistical difference between farms practicing particular vaccination programmes and farms not, in relation to the seropositivity against *Ornithobacterium rhinotracheale*. Although the vaccination programmes do

not include to confer immunity against *Ornithobacterium rhinotracheale*, the farms with the programmes would be more likely to practise the same or similar sanitary measures, which could contribute to prevent the chickens from various infection. Practice of a sanitary measure would be indicative of a poultry farm to distinguish potential risks of diseases. The farms practicing the vaccination programmes have the risk ratio of 0.3 for having seropositivity against *Ornithobacterium rhinotracheale*. This means those farms are 3.3 times (1/0.3) less likely to be classified as positive against this infectious agent, in comparison with the farms not practicing the same vaccination programmes. As the next step, seropositivity against the study infectious agents at individual-chicken level must be taken into further consideration.

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