ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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

International Journal of Poultry Science 9 (6): 533-536, 2010
ISSN 1682-8356

Prevalence of *Salmonella* Spp. in Backyard Chickens in Paraguay

G. Leotta[1], K. Suzuki[1], F.L. Alvarez[2], L. Nuñez[2], M.G. Silva[2], L. Castro[2], M.L. Faccioli[2], N. Zarate[3], N. Weiler[3], M. Alvarez[3] and J. Copes[1]
[1]Cátedra de Tecnología y Sanidad de los Alimentos,
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina
[2]Laboratorio de Diagnóstico de las Enfermedades de las Aves,
Facultad de Ciencias Veterinarias, Universidad Nacional de Asunción, Paraguay
[3]Departamento de Bacteriología y Micología, Laboratorio Central de Salud Pública,
Ministerio de Salud Pública y Bienestar Social, Paraguay

Abstract: The objective of this study was to (1) determine prevalence against *Salmonella* spp. and (2) investigate the risk factors with the positivity of the pathogens in backyard chickens in Paraguay. The field study was conducted between 31 March and 9 April 2009. A total of 50 smallholder farming households with chickens reared at backyards were initially selected from 25 of 52 administrative districts in San Lorenzo, Central Department, Paraguay. The required sample size of chickens was 400 in total. Data collection through questionnaire interviews about some selected poultry farming practices for each farm, together with cloacal swab sample collections for each chicken was implemented. The swab samples were examined microbiologically. Statistical analyses were used to describe the differences between the two chicken groups categorized according to positivity against *Salmonella* spp. The overall percentage of test-positive against *Salmonella* spp. was 3.5% [95% confidence interval (CI): 1.9%-5.7%]. The final logistic regression model indicated that free-range birds were more likely to have positivity against *Salmonella* spp., compared with caged birds (odds ratio: 3.5, 95% CI: 1.2-10.3).

Key words: IMS, logistic regression, risk factor, South America

INTRODUCTION

Salmonella spp. infections in poultry are comparatively common and have public health importance due to the consumption of contaminated poultry products (Vandeplas *et al*., 2010). *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella heidelberg* are amongst the most ordinary *Salmonella* infections in poultry, in spite of the fact that infections may be produced by 10-20 different serotypes (Carrique-Mas and Davies, 2008). Some species or strains are more pathogenic than others. The prevalence of other species differs widely spatially and temporally (O'Bryan *et al*., 2008). Transmission normally occurs horizontally from infected birds, contaminated environments, contaminated feed, or infected rodents (Babu and Raybourne, 2008). Except for *Salmonella enteritidis*, transmission of most serotypes to progeny from infected breeders is mostly through faecal contamination of the eggshell (Barrow, 2007). Infected chickens remain carriers (O'Bryan *et al*., 2008; Vandeplas *et al*., 2010). In Paraguay, several field investigations regarding the health of backyard chickens were conducted (Herrero *et al*., 2009; Origlia *et al*., 2009; Suzuki *et al*., 2009). However, no quantitative epidemiological investigations to determine prevalence against *Salmonella* spp. in backyard chickens and identify potential risk factors for the infection have been publicized to the best of the authors' knowledge. The objective of this study was to (1) determine prevalence against *Salmonella* spp. and (2) investigate the risk factors with the positivity of the pathogens in backyard chickens in Paraguay.

MATERIALS AND METHODS

Study area: Paraguay is a fully landlocked country with a land area of 406,752 km^2, located in the centre of South America, bordering Argentina to the south and southwest, Brazil to the east and northeast and Bolivia to the northwest. Paraguay consists of 17 departments and one capital district. The population is currently estimated at 6.2 million people, of which 3.6 million live in the capital city Asunción, its surroundings and other urban areas. The study area San Lorenzo is a city located in the Central Department in Paraguay, a satellite city to the east of Asunción. It is the third most populated city of this country. Paraguay has a poultry population of 21 million, a poultry meat production of 47,500 tones per year and a poultry egg production of 121,000 tones per year (FAO, 2010).

Sample collection: A total of 50 smallholder farming households with chickens reared at backyards were initially selected from 25 of 52 administrative districts in

Corresponding Author: K. Suzuki, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 60 y 118, CC296, La Plata, B1900AVW, Argentina

San Lorenzo, Central Department, Paraguay. The required sample size of 400 in total from a chicken population of 21 million was sufficient to obtain a 95% confidence interval (95% CI) with a desired precision of ±5% when the estimated prevalence was 50% (Hintze, 2008). The sample size of eight in each of the farms was equally assigned by the attainable financial, human and material means. The field study was conducted between 31 March and 9 April 2009, comprised data collection through questionnaire interviews for each farm selected, together with cloacal swab sample collections for each backyard chicken.

Laboratory examinations: For *Salmonella* isolation, Immunomagnetic Separation (IMS) was implemented according to the instruction manual (Dynal®, Invitrogen, Carlsbad, CA, USA), followed by use of xilose lysine tergitol 4 agar (XLT4). Suspect colonies were biochemically tested by Triple Sugar Iron (TSI) agar in combination with Lysine Iron Agar (LIA) to confirm the identity, according to the OIE's recommendations (OIE, 2009).

Data analysis: Data were entered into a database using the Base in the OpenOffice.org software version 3.2.1 (Sun Microsystems, Santa Clara, CA, USA). The statistical analyses were performed using Stata MP 11.1 (Stata Corporation, College Station, TX, USA). Univariate and multivariate analyses were used to describe the differences between the two chicken groups categorized according to positivity against *Salmonella* spp. Univariate analyses were conducted using Pearson's chi-squared statistic for categorical predictors such as "Age group" (Single versus Mixed), "Rearing place" (Caged vs. Free-range), "Feeding" (use of commercial balanced feed vs. homemade feed), "Water source" (Tap water vs. Well/stream), "Watering method" (ad-lib supplier vs. container put directly on the ground), "Use of medical treatment", "Ownership of animals other than chickens" and "Intrusion to the backyard by wildlife". Following the univariate analyses, a multivariate logistic regression analysis was conducted to better understand the relationships between the outcome "Positivity" and the predictors mentioned above. In the analysis, the most important predictors differentiating between the two chicken groups were identified, based on inclusion of all variables, which were significant at $p<0.1$ in the univariate analysis. A disadvantage of the univariate analysis was that a set of variables, of which each is weakly associated with the outcome, can become important predictors when they are taken together. To prevent this, a significance level that was relatively safe ($p<0.1$) was selected (Noordhuizen *et al*., 2001). A stepwise backward variable selection approach was used based on the likelihood-ratio statistic and removal probabilities of $p<0.05$. All variables included in the final regression model were screened for possible interactions (Katz, 1999).

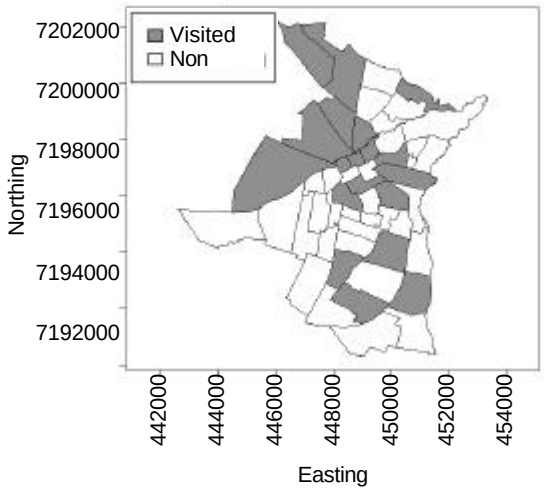

Fig. 1: A map showing the districts visited, for the prevalence study of *Salmonella* spp. in backyard chickens, in all the 52 administrative districts of San Lorenzo city, Central Department, Paraguay

RESULTS

Twenty-five different districts were selected (Fig. 1) and 48 farms were recruited (median number of the farms per district: one, range: one to five). The numbers of chickens reared between the farms were varied from three to 50 (median: 20). The median number of chickens selected between the farms was eight (range: one to 16). Among the study district the median number of chickens selected was 16 (range: 15-20). In total 405 chickens were studied, accounting for 43% of the study chicken population (N = 949) and 0.002% of the total chicken population in Paraguay. Cloacal swab samples from the chickens were examined. Fourteen out of 200 samples with suspect colonies by IMS followed by XLT4 were diagnosed as *Salmonella* spp. by TSI and LIA (positive predictive value of 7%, 95% CI: 4%-12%). The overall percentage of test-positive against *Salmonella* spp. was 3.5% (95% CI: 1.9%-5.7%). Regarding some selected poultry farming practices, 94% of the study farms used homemade feed for chickens. Ninety-eight percent of them used a drinking water container put directly on the ground for chickens (versus use of a funnelform ad-lib water supplier). Seventy-three percent of them had not given sick chickens any medical treatment such as anthelmintic and antibiotics. Other than chickens domestic animals such as dogs, cats and ducks were owned by 83% of them. Wild birds had an access to backyards at 98% of the farms. One farm responded to have found weasels at the backyard. No farms gave chickens vaccination against *Salmonella* spp. Table 1 shows descriptive statistics for percentage of test positive against *Salmonella* spp. in the study chickens corresponding to three variables with statistical

Table 1: Distribution of test-positive backyard chickens against *Salmonella* spp. in San Lorenzo city of Paraguay (with a selection criterion of p<0.1 for variables)

Variable	No. of chickens	% of test positive	Chi-squared statistic	df	P
Age group					
Mixed	375	2.9	4.2	1	0.041
Single	30	10.0			
Rearing state					
Caged	289	2.1	5.8	1	0.016
Free-range	116	6.9			
Water source					
Tap water	336	2.7	3.6	1	0.059
Well/stream	69	7.3			

Table 2: Final logistic regression model for risk of a backyard chicken in San Lorenzo city of Paraguay having test-positivity against *Salmonella* spp. in relation to a place where the chicken was reared (n = 405)

		95% confidence interval			
	Coefficient	Lower	Upper	Standard error	P
Caged (reference)					
Free-range	1.25	0.17	2.33	0.55	0.023
Constant	-3.85	-4.66	-3.05	0.41	<0.001

Likelihood ratio chi-squared statistic 5.1, df 1, p = 0.023

significance at p<0.1. These variables "Age group", "Rearing place" and "Water source" were included in the initial multivariate logistic regression model. The final model (Table 2) indicated that free-range birds were more likely to have positivity against *Salmonella* spp., compared with caged birds (odds ratio: 3.5, 95% CI: 1.2-10.3).

DISCUSSION

The results presented here constitute the first structured epidemiological approach carried out to estimate *Salmonella* spp. contamination in backyard chickens in San Lorenzo, Paraguay. In summary, during the period March - April 2009, the observed individual prevalence of *Salmonella* spp. in this study (3.5%) was similar to that reported by other authors in overseas (Wunderwald and Hoop, 2002; Namata *et al*., 2009; Aury *et al*., 2010). In fancy breed poultry flocks in Switzerland, Wunderwald and Hoop (2002) reported 10 and 4% of the samples serologically tested were positive for *Salmonella gallinarum-pullorum* and *Salmonella enteritidis*, respectively. Aury *et al*. (2010) reported the prevalence of *Salmonella* spp. positive flocks was 1.5% for breeding turkeys in France. Namata *et al*. (2009) reported caged and free-range chicken flocks in Belgium had positivity of *Salmonella* spp. of six and four percent, respectively. Although prevalence of several pathogens in backyard chickens in Latin America, where the present study area is located, was studied, *Salmonella* spp. was not reported (Hernandez-Divers *et al*., 2006; Hernandez-Divers *et al*., 2008). The study free-ranging chickens were three and a half times as likely to have positivity against *Salmonella* spp., in comparison with caged chickens. This interpretation would be supported by the findings that almost all of the study farms allowed wild birds to intrude the backyards. Pathogens of wild birds create a risk of disease for free-ranging chickens, as well as potential economic losses for resource-poor farmers. In comparison with commercial poultry, backyard chickens are both at an advantage and disadvantage for maintaining health. Backyard chickens are not given immunizations usually given to commercial poultry, involving vaccinating hens to raise maternal antibody passed to chicks. This makes backyard chickens intrinsically more sensitive to many infectious diseases. Commercial poultry are kept in single age groups in an "all in, all out" manner, while backyard chickens are usually in flocks of mixed ages, with susceptible chicks in touch with adults that are potential reservoirs for diseases. An infectious disease, therefore, could easily be maintained in a backyard chicken population by a constant supply of new susceptible hosts coming into contact with reservoir animals. The results of the present study, however, showed that Single age group was more likely to have positivity against *Salmonella* spp., in comparison with Mixed age group at univariate analysis (p = 0.041). This phenomenon is interesting and required further investigations. Although there was no statistical difference between the use of Tap water and Well/stream water (p = 0.059), watering not using tap water might be a risk factor for spreading *Salmonella* infection horizontally, as one of possible contaminated environments.

ACKNOWLEDGEMENTS

This study was carried out as part of the project for the capacity development for improvement of livestock hygiene in the southern part of South America through regional cooperation [commonly known as: Proyecto de desarrollo profesional continuo para los veterinarios del Sur (PROVETSUR)], funded by the Japan International Cooperation Agency.

REFERENCES

Aury, K., M. Chemaly, I. Petetin, S. Rouxel, M. Picherot, V. Michel and S. Le Bouquin, 2010. Prevalence and risk factors for *Salmonella enterica* subsp *enterica* contamination in French breeding and fattening turkey flocks at the end of the rearing period. Prev. Vet. Med., 94: 84-93.

Babu, U.S. and R.B. Raybourne, 2008. Impact of dietary components on chicken immune system and *Salmonella* infection. Expert Rev. Anti-Infect. Ther., 6: 121-135.

Barrow, P.A., 2007. *Salmonella* infections: Immune and non-immune protection with vaccines. Avian Pathol., 36: 1-13.

Carrique-Mas, J.J. and R.H. Davies, 2008. Sampling and bacteriological detection of *Salmonella* in poultry and poultry premises: A review. Rev. Sci. Tech. Off. Int. Epizoot., 27: 665-677.

FAO, 2010. FAOSTAT. Rome. Available from: http://faostat.fao.org/.

Hernandez-Divers, S.M., P. Villegas, C. Jimenez, S.J. Hernandez-Divers, M. Garcia, S.M. Riblet, C.R. Carroll, B.M. O'Connor, J.L. Webb, M.J. Yabsley, S.M. Williams and S. Sanchez, 2008. Backyard chicken flocks pose a disease risk for neotropic birds in Costa Rica. Avian Dis., 52, 558-566.

Hernandez-Divers, S.M., P. Villegas, F. Prieto, J.C. Unda, N. Stedman, B. Ritchie, R. Carroll and S.J. Hernandez-Divers, 2006. A survey of selected avian pathogens of backyard poultry in northwestern Ecuador. J. Avian Med. Surg., 20: 147-158.

Herrero, M., K. Suzuki, J. Origlia, L. Nuñez, M. Faccioli, M. Silva, J. Caballero, O.F. Valiente and Álvarez, 2009. Probability mapping for *Mycoplasma gallisepticum* risk in backyard chickens in Paraguay. Int. J. Poult. Sci., 8: 565-569.

Hintze, J., 2008. PASS 2008 software. NCSS, Kaysville, UT.

Katz, M.H., 1999. Multivariable analysis: A practical guide for clinicians. Cambridge University Press, Cambridge, pp: 192.

Namata, H., S. Welby, M. Aerts, C. Faes, J.C. Abrahantes, H. Imberechts, K. Vermeersch, J. Hooyberghs, E. Meroc and K. Mintiens, 2009. Identification of risk factors for the prevalence and persistence of *Salmonella* in Belgian broiler chicken flocks. Prev. Vet. Med., 90: 211-222.

Noordhuizen, J.P.T.M., K. Frankena, M.V. Thrusfield and E.A.M. Graat, 2001. Application of quantitative methods in veterinary epidemiology. Wageningen Pers, Wageningen, pp: 429.

O'Bryan, C.A., P.G. Crandall, S.C. Ricke and D.G. Olson, 2008. Impact of irradiation on the safety and quality of poultry and meat products: a review. Crit. Rev. Food Sci. Nutr., 48: 442-457.

OIE, 2009. Salmonellosis, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009. Paris. Available from: http://www.oie.int/eng/normes/mmanual/2008/pdf/2.09.09_SALMONELLOSIS.pdf.

Origlia, J., K. Suzuki, L. Castro, M. Faccioli, M. Silva, J. Caballero, O. Valiente and F. Álvarez, 2009. Bayesian mapping for infectious bronchitis virus risk in backyard chickens in Paraguay. Int. J. Poult. Sci., 8: 740-745.

Suzuki, K., J. Origlia, F. Álvarez, M. Faccioli, M. Silva, J. Caballero, L. Nuñez and L. Castro, 2009. Relative risk estimation for *Mycoplasma synoviae* in backyard chickens in Paraguay. Int. J. Poult. Sci., 8: 842-847.

Vandeplas, S., R.D. Dauphin, Y. Beckers, P. Thonart and A. Thewis, 2010. *Salmonella* in chicken: Current and developing strategies to reduce contamination at farm level. J. Food Prot., 73: 774-785.

Wunderwald, C. and R.K. Hoop, 2002. Serological monitoring of 40 Swiss fancy breed poultry flocks. Avian Pathol., 31: 157-162.