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Relative Risk Estimation for *Mycoplasma synoviae* in Backyard Chickens in Paraguay

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Abstract: Poultry production is a growing industry in Paraguay, in southern South America. The insufficient farm management methods frequently applied in backyard chickens make them a possible reservoir for economically important diseases such as *Mycoplasma synoviae* that can influence commercial poultry operations. There are no former studies on a survey of *Mycoplasma synoviae* among backyard chickens in Paraguay. The objectives of this study were: (1) to observe the seroprevalence of MS in backyard chickens in Paraguay and (2) to generate maps for the estimated Relative Risk (RR) for MS in the study chickens, in place of using the observed seroprevalence. Paraguay is divided into 17 departments. A department-stratified random sampling was arranged and conducted. The required total sample size of 1537 from a chicken population of 17 million was sufficient to produce a 95% confidence interval with a desired precision of ±2.5% when the estimated seroprevalence was 50%. Sera were examined using a commercial indirect ELISA. The overall observed seroprevalence was 53%. The resulting maps for the estimated RR for *Mycoplasma synoviae* in the study chickens at department level were drawn. Departments with notably high or low disease risks were confirmed. Different types of epidemiological parameters can be calculated to take account of probable risk factors. Therefore, additional detailed investigations into those risk factors relating to *Mycoplasma synoviae* occurrence with respect to spatially epidemiological dissimilarities would be of interest.

Key words: Choropleth map, data visualization, respiratory disease

INTRODUCTION

Mycoplasma Synoviae (MS) is a considerable and economically crucial pathogen of avian species. The MS is the etiological agent of acute to chronic respiratory disease in chickens and may be horizontally and vertically infected. Chickens infected with MS become sneezing nasal discharge, foam in eye, rattle breathing and swollen sinus (Kleven, 2003; 2005). Ever more, these possible performance and production deprivations require the harsh control of MS amongst all the poultry sectors.

Poultry production is a growing field in Paraguay. A number of commercial poultry operations have been developed in the vicinity of some urban areas. The insufficient farm management methods frequently applied in backyard chickens make them a possible reservoir for diseases such as MS that can influence commercial poultry operations. Investigations into the diseases including MS of backyard chickens in the world are infrequent (McBride *et al.*, 1991; Kelley *et al.*, 1994; Hernandez-Divers *et al.*, 2006), but should be encouraged. There are no former studies, as far as the authors know, on a MS survey among backyard chickens in Paraguay.

Incidentally, the most commonly used spatially epidemiological analysis technique in studies of animal diseases is data visualization. This includes generating maps to show the spatial patterns of disease occurrence, which are then used to make hypotheses about potential cause-effect associations. While a choropleth map showing the proportion classified as test positive of a disease (e.g. observed seroprevalence) is easy to understand, it has disadvantage that the size of the areas and the location of their borders is typically an indication of administrative requirements rather than of the geographical distribution of epidemiological factors. The objectives of this study were: (1) to observe the seroprevalence of MS in backyard chickens in Paraguay and (2) to generate maps for the estimated Relative Risk (RR) for MS in the study chickens, in place of using the observed seroprevalence.

MATERIALS AND METHODS

Study area: Paraguay is a fully landlocked country with a land area of 406,752 km^2, located in southern South America, bordering Argentina to the south and southwest, Brazil to the east and northeast and Bolivia to the northwest. Paraguay is divided into 17 departments and one capital district. The population is considered at 6.2 million people, of which 3.6 million reside in the capital city Asunción, its surroundings and other urban areas. About 40% of the entire population engages in agricultural sector. The climate in Paraguay is subtropical with mean monthly temperature varying from 18°C in winter to 28°C in summer. Mean annual rainfall ranges between 750 mm and 1250 mm, increasing southwards. Paraguay owns a poultry population of 17 million, a poultry meat production of 37,000 tonnes per year and a poultry egg production of 100,000 tonnes per year (FAO, 2001; 2005; 2009).

Data and sample collections: The Paraguay government's Statistical Yearbook 2006 used for this study contained data on the number of chickens at department level (DGEEC, 2008). A map of administrative boundaries was got from the GIS Download Data Server (CIP, 2005). A choropleth map of Paraguay on the basis of the population density of chickens per square kilometre at department level was produced using the geographical information system software ArcGIS version 9.2 (ESRI Inc., Redlands, CA, USA). All the departments were classified into five groups, based on the number of chickens per square kilometre using built-in Natural Breaks (Jenks) function in the software with a minor modification: less than 20, 21-50, 51-150, 151-250 and 251 and over.

The Paraguay government's National Animal Quality and Health Service [Servicio Nacional de Calidad y Salud Animal (SENACSA)] arranged a department-stratified random sampling, originally for a nationwide survey for Newcastle Disease and Avian Influenza in backyard chickens in Paraguay (SENACSA, 2006). The required total sample size of 1537 from a chicken population of 17 million was sufficient to produce a 95% confidence interval (CI) with a desired precision of ±2.5% when the estimated seroprevalence was 50%. The sample size in each of the departments was allocated by the accessible financial, human and material means. The field investigation was conducted in 2006, consisted of data collection through questionnaire interviews for each smallholder farming household, in conjunction with blood sample collections for each backyard chicken.

Laboratory examinations and data analysis: Sera imparted by SENACSA were examined using a commercial indirect ELISA (FlockChek® MS, IDEXX Laboratories Inc., Westbrook, ME, USA) as indicated by the manufacturer instruction for detecting antibodies against MS, between March and August 2008. Absorbance was read on an ELISA reader with wavelength at 650 nm. A level of antibody titres larger than 1076 was considered positive. Given the finite use of vaccine against any diseases in the backyard chicken population in Paraguay, most of the results of laboratory examinations would be ascribed to natural exposure to MS (SENACSA, 2006, personal communication). After acquiring the results of laboratory examinations, power analysis for the overall proportion classified as test positive (observed seroprevalence) was carried out using the statistical power analysis software PASS 2008 version 08.0.8 (Number Cruncher Statistical Systems, Kaysville, UT, USA). CIs of observed seroprevalence in each of the departments were calculated by Wilson's method (Newcombe, 1998). The ELISA sensitivity and specificity values were not publicised and therefore further computations for true prevalence estimation (Rogan and Gladen, 1978) were not implemented.

The estimated number of positives in each of the departments y_i was obtained by multiplying the governmentally reported chicken population in a department n_i by the corresponding observed seroprevalence. The overall observed seroprevalence was presumed in fact constant for all departments and those departments were independent. Then a sensible supposition was that estimated number of positives y_i were observations on independent Poisson random variables with expected values e_i. In this case a reasonable estimate of the mean expected number in each department, e_i, was given by:

$$\hat{e}_i = n_i \left(\frac{\sum y_i}{\sum ni} \right)$$

The standardized relative risk (SRR) $\hat{\theta}_i$ in each of the departments was calculated by dividing the estimated number of positives y_i by its estimated expected value, e_i (Dohoo *et al*., 2003):

$$\hat{\theta}_i = SRR_i = \frac{y_i}{e_i}$$

The numbers of seropositives in each department were assumed to be mutually independent and to follow Poisson distributions:

$$y_i \sim Poisson(e_i \theta_i) \, \forall i$$

A gamma prior distribution for the RRs combined conveniently with the Poisson likelihood to give a gamma posterior distribution. If the prior distribution was a Gamma (a, b), the RR had the following posterior distribution:

$$Gamma(a + y_i, b + e)$$

with mean given by:

$$E\left[\theta_i \middle| y_i, a, b\right] = \frac{a + y_i}{b + e_i}$$

$$= \omega_i SRR_i + (1 - \omega_i) \frac{a}{b}$$

where $\omega_i = e_i/b+e_i$. The posterior mean of the RR for the ith department was a weighted average of the SRR for the ith department and the RR in the overall map, the weight being inversely related to the variance of the SRR. For rare diseases and small areas, this variance is large so the weight ω_i, is small and the posterior mean tends towards a global mean a/b, thereby producing a smoothed map. In departments with abundant data the posterior mean of the RR is close to y_i/e_i. Markov Chain-Monte Carlo (MCMC) estimates of parameters were obtained for the Poisson-gamma model described above. The MCMC simulation was run for 110,000 iterations of which the first 10,000 iterations were discarded as 'burn-in'. The models were run in the WinBUGS software version 1.4.3 (Lunn *et al*., 2000). The posterior means and 95% Bayesian Credible Interval (BCI) were recorded for each estimate. The resulting maps for the posterior means and 95% BCIs were drawn using the maptools package version 0.7-20, in the R software version 2.8.1 (R Development Core Team, 2008; Lewin-Koh and Bivand, 2009).

RESULTS

Figure 1 shows a map showing the population density of chickens per square kilometre in 17 departments of Paraguay. The capital city Asunción, where is a distinct administrative area from any other departments, is actually situated in Central Department. The Central Department had the largest chicken population density of 1626/km². The east side of the country had the concentration of chicken population, mainly in the capital's surroundings and the vicinity of the other urban areas such as Ciudad del Este in Alto Paraná Department and Encarnación in Itapúa Department. While the three departments in the west side of the country (Presidente Hayes, Boquerón and Alto Paraguay) where were scarsely populated areas (< 2 people/km²) had less chicken population. The Alto Paraguay Department had the lowest chicken population density of <1/km². Table 1 represents descriptive results. The statistical precision was improved from ±2.5% to ±2.4% because of the eventual total number of samples of 1743 (larger than planned) and the overall observed seroprevalence of 53% (larger than expected). Observed seroprevalence was highly variable between the study departments (8-90%) on the basis of different sample sizes (33-292). The median number and range of the study households per department and chickens per household were 19 (range: 5-40) and 9 (range: 1-29), respectively. The estimated seroprevalence were larger and smaller than each of the observed seroprevalence for the nine and six departments, respectively. The resulting map for the posterior means is shown in Fig. 2. All the departments were classified into five groups, on the basis of the SRR. Small (0.2-0.5) or large (1.5-1.7) SRRs indicate that the study departments have

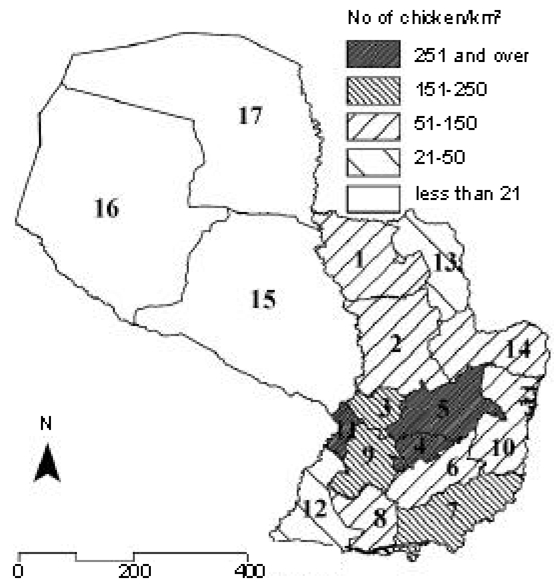

Fig. 1: A choropleth map based on the population density of chickens per square kilometre in 17 administrative departments of Paraguay. The departmental IDs (1-17) refer to values shown in (Table 1)

significantly lower (Departments of Ñeembucú and alto Paraguay) or higher risk (Departments of Guairá and Paraguarí), respectively. The resulting maps for the 95% BCI are depicted in Fig. 3. In the map for the lower 95% Bayesian Credible Limit (BCL), Departments of Concepción and Alto Paraguay are found that their SRR levels go down a notch in comparison with the map for the posterior means. The map for the upper 95% BCL has the same visual information as the map for the posterior means in Fig. 2.

DISCUSSION

This study showed the utility of serum samples of backyard chickens officially collected, for analysis of spatial characteristics of MS risk in Paraguay. The dataset supplied information on the results of the diagnostic test for MS, allowing analysis of individual-based data for the study. The moderate-sized dataset posed a few computational challenges and as a result, some analytical compromises had to be implemented. For example, the application of sampling weight correction techniques to the stratified survey dataset using the survey package version 3.11-2 in the R software (Lumley, 2004; 2009), would have been desirable, but was computationally impossible. Seroprevalence at flock level was also not taken into consideration. In spite of the limitations, it is considered that the analysis has provided an accurate description of the spatial characteristics of MS risk in Paraguay.

Table 1: Observed and estimated seroprevalence for *Mycoplasma synoviae* in backyard chickens in Paraguay

ID	Department	No. of samples	Observed seroprevalence [%, (95% CI)]	Estimated seroprevalence [%, (95% BCI)]
1	Concepción	56	60.7 (47.6-72.4)	61.0 (60.9-61.2)
2	San Pedro	104	67.3 (57.8-75.6)	67.0 (66.9-67.1)
3	Cordillera	139	30.2 (23.2-38.3)	30.0 (29.9-30.1)
4	Guairá	127	7.87 (4.33-13.9)	8.00 (7.96-8.05)
5	Caaguazú	166	71.1 (63.8-77.4)	71.0 (70.9-71.1)
6	Caazapá	86	68.6 (58.2-77.4)	69.0 (68.9-69.1)
7	Itapúa	173	34.1 (27.5-41.4)	34.0 (33.9-34.1)
8	Misiones	50	90.0 (78.6-95.7)	90.0 (89.7-90.3)
9	Paraguarí	99	27.3 (19.5-36.8)	27.0 (26.9-27.1)
10	Alto Paraná	149	49.7 (41.7-57.6)	50.0 (49.9-50.1)
11	Central	292	60.6 (54.9-66.0)	61.0 (60.9-61.1)
12	Ñeembucú	76	82.9 (72.9-89.7)	83.0 (82.7-83.3)
13	Amambay	48	62.5 (48.4-74.8)	63.0 (62.8-63.3)
14	Canindeyú	36	52.8 (37.0-68.0)	53.0 (52.9-53.1)
15	Presidente Hayes	50	62.0 (48.2-74.1)	62.0 (61.6-62.4)
16	Boquerón	33	69.7 (52.7-82.6)	70.0 (69.4-70.6)
17	Alto Paraguay	59	78.0 (65.9-86.6)	78.0 (76.4-79.6)
Total		1743	53.2 (50.8-55.5)	51.6 (51.6-51.6)

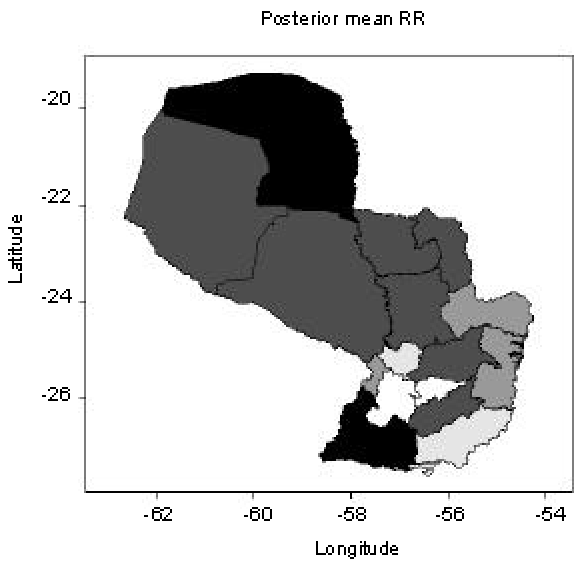

Fig. 2: Choropleth map showing posterior mean Relative Risk (RR) for *Mycoplasma synoviae* in backyard chickens in each department in Paraguay

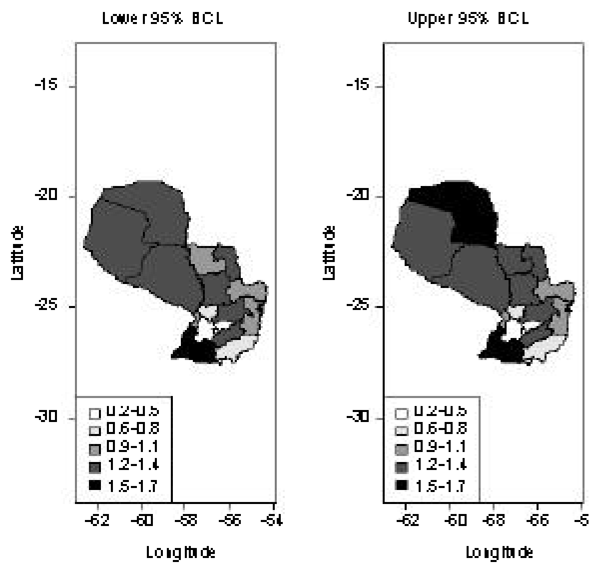

Fig. 3: Choropleth map showing lower and upper 95% Bayesian Credible Limits (BCL) of relative risk for *Mycoplasma synoviae* in backyard chickens in each department in Paraguay

In this study, backyard chickens in Paraguay demonstrated evidence of exposure to MS, one of important poultry pathogens. Canindeyú Department had the same observed seroprevalence value of 53% as the value of the whole study area. The overall observed seroprevalence approximately agreed with the prevalence in backyard chickens (McBride *et al.*, 1991; Kelley *et al.*, 1994; Hernandez-Divers *et al.*, 2006) and fancy breeding chickens (Wunderwald and Hoop, 2002) where flock management methods are comparable with backyard chickens.

In comparison with commercial poultry, backyard chickens are both at an advantage and disadvantage for maintaining health. Backyard chickens are not given immunisations usually given to commercial poultry, including vaccinating hens to raise maternal antibody passed to chicks. This makes backyard chickens intrinsically more sensitive to numerous infectious diseases. In addition, backyard chickens do not receive treatments commonly used in commercial poultry, such as coccidiostat drugs (SENACSA, 2006, personal communication). Commercial poultry are bred in single age groups in an "all in, all out" manner, while backyard chickens are in flocks of mixed ages, with sensitive 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 birds. The relatively high seroprevalence in some departments can be explained in part by repeated stimulation from close contact with bacteria due to the common rearing of various age groups. Most commercial poultry flocks are kept free of certain contagious diseases including MS, which can be transmitted from the hen to her progeny (SENACSA, 2006, personal communication). As backyard chickens are not kept under monitoring for the diseases, diseases would remain endemic in the population through continued egg transmission.

When the attribute of interest is a proportion (e.g. observed seroprevalence), exploratory mapping of the proportions to present spatial features is a clear first measure in any analysis. However, using the raw observed proportions may be misleading, because the variability of such proportions will be a dependence on the values of the population to which they relate and this may differ broadly between areas (Bailey and Gatrell, 1995). An alternative practice for evaluating these data would be to focus on the differences between the departments and national average in the case of this study. The SRR is utilized in representing spatial characteristics of disease distribution. It systematizes the data by re-expressing them as the ratio between the estimated number of positives and the number that would have been expected in a standard population. Figure 2 shows SRR estimates for MS-positive backyard chickens aggregated at department level. This is mostly a dependence on sample size and it is therefore suitable to accompany the map with a presentation of the probability of the estimates, such as Fig. 3.

As the objective of these map presentations is to identify departments with uncommonly high or low disease risks, different types of epidemiological parameters could be calculated taking account of potential risk factors, such as the spatial heterogeneity of the underlying population at risk. Therefore, further detailed studies into those risk factors associated with MS occurrence with respect to spatially epidemiological differences would be of interest.

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