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## Bayesian Mapping for Infectious Bronchitis Virus Risk in Backyard Chickens in Paraguay

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**Abstract:** Poultry production is a developing industry in Paraguay, South America. The inadequate farm management methods commonly used in backyard chickens make them a potential reservoir for economically important diseases such as infectious bronchitis that can affect commercial poultry operations. There are no earlier studies on a survey of Infectious Bronchitis Virus (IBV) amongst backyard chicken population in Paraguay. The objectives of this study were: (1) to observe the seroprevalence of IBV in backyard chickens in Paraguay and (2) to generate maps for estimated seroprevalence using Bayesian techniques for IBV in the study chickens and for smoothed Relative Risks (RR) drawn by one of the techniques above, in place of using the observed seroprevalence. Paraguay consists of 17 departments. A department-stratified random sampling was designed and implemented. The required total sample size of 1537 from a chicken population of 17 million was adequate to produce a 95% confidence interval with a desired precision of  $\pm 2.5\%$  when the estimated seroprevalence was 50%. Sera were analyzed using a commercial indirect ELISA. The overall observed antibody seroprevalence was 80%. The resulting maps for the estimated seroprevalence for IBV using Bayesian techniques in the study chickens at department level, and for the smoothed RRs were illustrated. Different types of epidemiological parameters can be computed to take account of potential risk factors. Therefore, further detailed studies into those risk factors associated with IBV appearance with respect to spatially epidemiological variations would be of interest.

**Key words:** Choropleth map, data visualization, smallholders

### INTRODUCTION

Infectious Bronchitis Virus (IBV) is a significantly and economically important pathogen of avian species. The IBV is the etiological agent of acute respiratory disease in chickens and is worldwide in distribution. Chickens infected with IBV become lessening in egg quality, feed conversion and growth rate and condemnation of meat at processing (Cavanagh and Naqi, 2003; Naqi, 2005). Ever more, these possible performance and production deprivations need the severe control of IBV among all the poultry sectors.

Poultry production is a developing industry in Paraguay. Several commercial poultry operations have been expanded in the suburbs of some urban areas. The inadequate farm management methods commonly used in backyard chickens make them a potential reservoir for diseases such as IBV that can affect commercial poultry operations. Field investigations into the diseases including IBV of backyard chickens in the world are relatively rare (McBride *et al.*, 1991; Kelley *et al.*, 1994; Hernandez-Divers *et al.*, 2006), but should be encouraged. There are no earlier studies, as far as the authors know, on an IBV survey amongst backyard chicken population in Paraguay.

By the way, the most commonly used spatial analysis technique in surveys of animal diseases is data

visualization. This includes making maps to display the spatial patterns of disease occurrence, which are then utilized to establish hypotheses about potential cause-effect associations. While a choropleth map illustrating the proportion classified as test positive of a disease (e.g. observed seroprevalence) is easy to comprehend, it has drawback that the size of the areas and the location of their borders is normally a manifestation of administrative requirements rather than of the geographical distribution of epidemiological components. The objectives of this study were: (1) to observe the seroprevalence of IBV in backyard chickens in Paraguay and (2) to generate maps for estimated seroprevalence using Bayesian techniques for IBV in the study chickens and for smoothed Relative Risks (RR) drawn by one of the techniques above, in place of using the observed seroprevalence.

### MATERIALS AND METHODS

**Study area:** Paraguay is a landlocked country with a land area of 406,752 km<sup>2</sup>, located in the centre of South America. It is bordered on 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. Currently the population is estimated at 6.2 million people, of which

3.6 million live in the capital city Asunción, its suburbs and other urban areas. Some 40% of the total population work in agricultural sector. Its climate is subtropical with average monthly temperature varying between 18°C in winter and 28°C in summer. Average annual rainfall ranges from 750-1250 mm, increasing southwards. The country has 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 utilized for this study contained data on the number of chickens at department level (DGEEC, 2008). A map of administrative boundaries was acquired from the GIS Download Data Server (CIP, 2005). A choropleth map of Paraguay based on the population density of chickens per square kilometer at department level was generated using the geographical information system software ArcGIS version 9.2 (ESRI Inc., Redlands, CA, USA). All the departments were categorized into 5 groups, based on the number of chickens per square kilometer using built-in Natural Breaks (Jenks) function in the software with a minor modification:  $\leq 20$ , 21 - 50, 51 - 150, 151-250 and  $\geq 251$ .

The Paraguay government's National Animal Quality and Health Service [Servicio Nacional de Calidad y Salud Animal (SENACSA)] designed 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 adequate to produce a 95% Confidence Interval (CI) with a desired precision of  $\pm 2.5\%$  when the estimated seroprevalence was 50%. The sample size in each of the departments was decided by the available financial, human and material resources. The field investigation was implemented in 2006, consisted of data collection through questionnaire interviews for each smallholder farming household, together with blood sample collections for each backyard chicken reared.

**Laboratory examinations and data analysis:** Sera provided by SENACSA were analyzed using a commercial indirect Enzyme-linked Immunosorbent Assay (ELISA) (FlockChek<sup>®</sup> IBV, IDEXX Laboratories Inc., Westbrook, ME, USA) as stated by the manufacturer instruction for detecting antibodies against IBV, from March to October 2008. Absorbance was read on an ELISA reader at 650 nm. A level of antibody titres greater than 396 was classified positive. Assuming the limited use of vaccine against any diseases in the backyard chicken population in Paraguay, most of the results of laboratory examinations would be attributed to natural exposure to IBV (SENACSA, 2006, personal

communication). After getting the results of laboratory tests, power analysis for the overall 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 computed using Wilson's method (Newcombe, 1998). The ELISA sensitivity and specificity values were not published and therefore further estimations for true prevalence (Rogan and Gladen, 1978) were not conducted.

The estimated number of positives in each department  $y_i$  was obtained by multiplying the governmentally published chicken population in a department  $n_i$  by the corresponding observed seroprevalence  $r_i$ .

(1) Using the moments procedure, we preliminary estimated mean value  $\gamma$  by the pooled mean of the observed seroprevalence, that was:

$$\hat{\gamma} = \frac{\sum y_i}{\sum n_i}$$

and to estimate variance  $\phi$ , based on a weighted sample variance of observed seroprevalence about this mean as:

$$\hat{\phi} = \frac{\sum n_i (r_i - \hat{\gamma})^2}{\sum n_i} - \frac{\hat{\gamma}}{\bar{n}}$$

where  $\bar{n}$  is the average population across all the departments and the convention was adopted that  $\hat{\phi} = 0$  whenever the above expression is negative. With these estimates  $\hat{\gamma}$  and  $\hat{\phi}$ , the shrinkage weighting factor was estimated as:

$$\hat{w}_i = \frac{\hat{\phi}}{\hat{\phi} + \frac{\hat{\gamma}}{n_i}}$$

and the Bayes estimates of the seroprevalence were:

$$\hat{\theta}_i = \hat{\gamma} + \frac{\hat{\phi}_i (r_i - \hat{\gamma})}{\hat{\phi} + \frac{\hat{\gamma}}{n_i}}$$

(2) Using the Maximum Likelihood (ML) procedure, we supposed that the true seroprevalence in each department was  $\theta_i$ . We also supposed that we had a prior probability distribution for each  $\theta_i$ , with mean value  $\gamma$  and variance  $\phi_i$ . It could be shown that the best Bayes estimates of  $\theta_i$ , based on combining these prior

distributions with the observed seroprevalence were given by:

$$\hat{\theta}_i = w_i r_i + (1 - w_i) \gamma_i$$

where,

$$w_i = \frac{\hat{\phi}_i}{\hat{\phi}_i + \gamma_i / n_i} \text{ (known as a shrinkage estimate)}$$

We give increasing weight to the observed seroprevalence for department  $i$  as  $w_i$  approaches 1, while the estimate for  $i$  effectively approaches the prior mean  $\gamma_i$  as  $w_i$  approaches 0. It becomes possible to derive ML estimates for  $\gamma$  and  $\phi$  from the observed seroprevalence, if we assume some mathematical form for the prior distribution, of which these are the mean and variance, such as gamma distribution which has two parameters,  $\nu$  and  $\alpha$ . The mean of distribution is given as  $\nu/\alpha$  ( $= \gamma$ ) and the variance as  $\nu/\alpha^2$  ( $= \phi$ ). Given such estimates  $\hat{\nu}$  and  $\hat{\alpha}$ , the estimated weighting factor in our earlier expression for the shrinkage estimator was:

$$\begin{aligned} \hat{w}_i &= \frac{\hat{\phi}}{\hat{\phi} + \frac{\hat{\gamma}}{n_i}} \\ &= \frac{\hat{\nu} / \hat{\alpha}^2}{\frac{\hat{\nu}}{\hat{\alpha}^2} + \frac{\hat{\nu}}{n_i \hat{\alpha}}} \\ &= \frac{n_i}{n_i + \hat{\alpha}} \end{aligned}$$

and the Bayes estimates of the seroprevalence were:

$$\begin{aligned} \hat{\theta}_i &= \hat{w}_i r_i + (1 - \hat{w}_i) \hat{\gamma} \\ &= \hat{w}_i r_i + \frac{(1 - \hat{w}_i) \hat{\nu}}{\hat{\alpha}} \\ &= \frac{y_i + \hat{\nu}}{n_i + \hat{\alpha}} \end{aligned}$$

When  $n_i$  is large  $w_i$  is close to 1 and so most weight in the estimate of  $\theta_i$  is given to  $r_i$ . When  $n_i$  is small more weight is given to  $\hat{\nu}/\hat{\alpha}$ , our estimate of the prior mean. The smoothed RRs were  $[O_i + \nu] / [E_i + \alpha]$ , where  $O_i$  and  $E_i$  were observed and expected number of cases for department  $i$ , respectively. Calculations using the moments and ML procedure were done using the spdep package version 0.4-34 and DCluster package version 0.2-2 in the R software version 2.8.1 (R Development Core Team, 2008; Bivand, 2009; Gómez-Rubio *et al.*, 2009). The resulting maps for comparing the estimated seroprevalence with observed seroprevalence in each department by using the two different procedures mentioned above and for the smoothed RR using ML procedure were drawn using the mapproj package version 0.7-20 in the R software (Lewin-Koh and Bivand, 2009).

## RESULTS

Figure 1 illustrates a map showing the population density of chickens per square kilometer in 17 departments of Paraguay. The capital city Asunción, where is a specific administrative zone from any other departments, is in fact located in Central Department. The Central Department had the highest chicken population density of 1626/km<sup>2</sup>. The east side of the country possessed the cluster of chicken population, especially in the capital's surroundings and around the other urban areas such as Encarnación in Itapúa Department and Ciudad del Este in Alto Paraná Department. While the three departments in the west side of the country (Presidente Hayes, Boquerón and Alto Paraguay) where were infrequently populated areas (<2/km<sup>2</sup>) had less chicken population. The Alto Paraguay Department had the lowest chicken population density of <1/km<sup>2</sup>. Table 1 represents descriptive results. The statistical precision was improved from  $\pm 2.5\%$  to  $\pm 1.9\%$  because of the eventual total number of samples of 1746 (larger than planned) and the overall observed seroprevalence of 80% (larger than expected). Observed seroprevalence was particularly varying between the study departments (54-99%) based on 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 mean and variance estimates were  $\hat{\gamma} = 0.827$  and  $\hat{\phi} = 0.012$  by the moments procedure and  $\hat{\gamma} = 0.939$  and  $\hat{\phi} = 0.035$  by the ML procedure. The resulting maps for comparing the estimated seroprevalence and observed seroprevalence in each department by using the two different estimating procedures above are shown in Fig. 2. The estimated seroprevalence by the moments procedure for the nine departments in the order of increasing observed seroprevalence from the lowest were larger than each of the observed seroprevalence. Meanwhile, the estimated seroprevalence by the ML procedure for all the departments except for Guairá Department which had the highest observed seroprevalence were larger than each of the observed seroprevalence. The choropleth map showing the smoothed RR by ML procedure is depicted in Fig. 3. There was no department with a probability of statistical insignificance. Small (<0.9) or large (>1.1) RRs indicate that the study departments have significantly lower (the number of departments applicable = 7) or higher risk (the number of departments applicable = 7), respectively, with three exceptional departments of Itapúa, Alto Paraná and Central having the RR of just 1.0.

## DISCUSSION

This study gave a demonstration of the utility of sera of backyard chickens officially collected, for spatial analysis on characteristics of IBV risk in Paraguay. The modest-

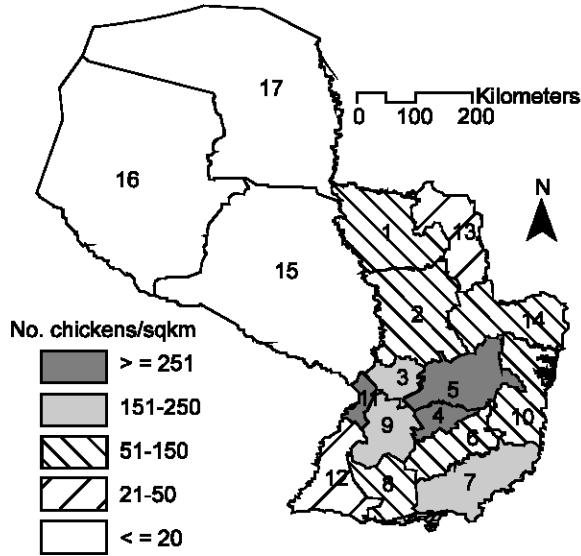


Fig. 1: A choropleth map based on the population density of chickens/km<sup>2</sup> in 17 administrative departments of Paraguay. The departmental IDs (1-17) refer to values shown in Table 1

sized dataset produced a few calculational challenges and consequencely, some analytical compromises had to be required. For example, the application of sampling weight rectification methods to the classified survey dataset using the survey package version 3.11-2 in the R software (Lumley, 2004, 2009), would have been desired, but was not computationally possible. Seroprevalence at flock level was also not dealt with. In spite of those limitations, it is considered that the analysis has supplied a precise description of the spatial features of IBV risk in Paraguay. In this study, backyard chickens in Paraguay gave evidence of exposure to IBV, one of significant poultry

pathogens. Alto Paraná and Central Departments had the same observed seroprevalence value of 80% as the value of the whole study area. The overall observed seroprevalence approximately agreed on 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 is comparable with backyard chickens. In comparison with commercial poultry, backyard chickens are both at a benefit and drawback for maintaining health. Backyard chickens are not often administered immunisations usually done to commercial poultry, involving vaccinating hens to increase maternal antibody passed to chicks. This makes backyard chickens inherently more susceptible to various infectious diseases. Additionally, backyard chickens do not acquire treatments regularly 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” method, while backyard chickens are in flocks of varied ages, with sensitive chicks in touch with adult chickens that are possible reservoirs for diseases. Therefore, an infectious disease could easily be kept in a backyard chicken population by a continuous supply of new susceptible hosts coming into contact with reservoir birds. The relatively high seroprevalence in some departments can be partly interpreted by repeated stimulation from close contact with viruses due to the common rearing of mixed age groups. Most commercial poultry flocks are preserved free of certain infectious diseases including IBV, which can be transmitted by airborne droplets, ingestion of contaminated feed and water and contaminated equipment (SENACSA, 2006, personal communication). Because backyard chickens are not bred under observation for the diseases, diseases would persist endemic in the population.

Table 1: Observed seroprevalence for infectious bronchitis virus in backyard chickens in Paraguay

ID	Department	No. of samples	Observed seroprevalence (%)	----- 95% CI -----	
				Lower	Upper
1	Concepción	61	61	48	72
2	San Pedro	104	90	83	95
3	Cordillera	139	73	65	79
4	Guairá	127	99	96	100
5	Caaguazú	166	92	86	95
6	Caazapá	88	64	53	73
7	Itapúa	171	85	79	89
8	Misiones	50	54	40	67
9	Paraguarí	93	89	81	94
10	Alto Paraná	152	80	73	85
11	Central	292	80	75	84
12	Ñeembucú	76	67	56	77
13	Amambay	48	92	80	97
14	Canindeyú	36	92	78	97
15	Presidente Hayes	52	46	33	59
16	Boquerón	33	67	50	80
17	Alto Paraguay	58	90	79	95
Total		1746	80	78	82

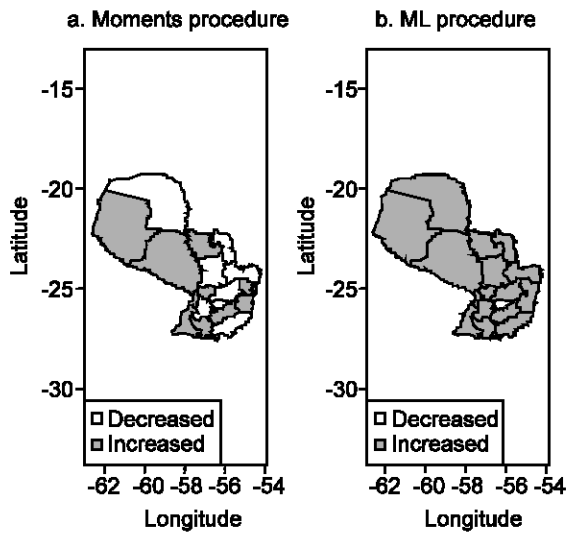


Fig. 2: Choropleth maps showing the comparison of the estimated seroprevalence with observed seroprevalence for infectious bronchitis virus in backyard chickens in Paraguay, by using (a) moments procedure and (b) maximum likelihood procedure. A department was filled with white in colour when the estimated seroprevalence was smaller than observed seroprevalence, while filled with grey in colour when was larger

When the attribute of interest is a proportion (e.g. observed seroprevalence), explorative mapping of the proportions to display spatial diversity is a clear first method in any analysis. Using the raw observed proportions, however, may be misleading, because the variability of such proportions will be a dependence on the values of the population to which they associate and this may differ broadly between areas (Bailey and Gatrell, 1995). The alternative to mapping observed values is the estimation and mapping, of a measure of RR. Bayesian estimation techniques are concerned with statistical estimation where prior knowledge or beliefs about parameters of interest are taken into account when estimating their values, as well as observed data. The maps of the Bayes estimates of the IBV seroprevalence based on the moments and ML estimates are shown in Fig. 2. In this case, there is a difference in the results from either method based on the different mean and variance estimations. In the moments method, lower seroprevalence in nine departments increased towards the overall mean. Meanwhile higher seroprevalence in the other eight departments was shrunk to a lesser degree. Figure 3 shows the map of the posterior expected RRs for each department based on the Poisson-gamma model. We are now dealing with a smoother map with less extremes in the RR estimates.

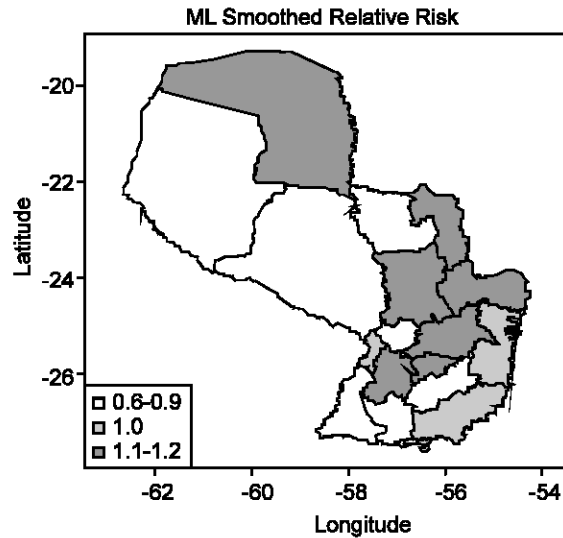


Fig. 3: Choropleth map showing the smoothed relative risk for infectious bronchitis virus in backyard chickens in Paraguay, calculated by Maximum Likelihood (ML) procedure

As the objective of these map productions is to distinguish departments with unexpectedly high or low disease risks, different types of epidemiological parameters can be computed to take 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 IBV appearance with respect to spatially epidemiological variations would be of interest.

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