# STOCHASTIC ESTIMATION FOR SEROPREVALENCE OF INFECTIOUS LARYNGOTRACHEITIS VIRUS IN BROILERS IN URUGUAY

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***ABSTRACT:*** *The objective of this study was to estimate the true seroprevalence of seropositive individual broilers against infectious laryngotracheitis virus in Uruguay using a Bayesian inference software based on Markov chain Monte Carlo technique. Seventeen farms were kept under investigation between 2008 and 2009. Each study flock was randomly selected at different farms recruited from the capital city Montevideo, Canelones and Lavalleja Departments. The required total sample size was determined by power analysis, and blood samples collected were analysed using a commercial ELISA for the detection of antibody to the pathogen mentioned above. The overall seroprevalence of the virus was estimated at 31.5% [95% Bayesian credible interval (16.8–49.2%); N = 1790]. Because none of the study broilers had been inoculated against the virus prior to sampling, most of these results could be ascribed to natural exposure by field viruses and/or vaccine viruses from neighbouring layers. It should be considered as further risk assessment for clarifying the suitable vaccines to prevent chicken population in Uruguay from the virus.*

**KEY WORDS:** epidemiology, herpesvirus, poultry

# ESTIMACION ESTOCASTICA DE LA SEROPREVALENCIA DEL VIRUS DE LARINGOTRAQUEITIS INFECCIOSA EN POLLOS PARRILLEROS DE URUGUAY

***RESUMEN****: El objetivo de este estudio fue estimar la real seroprevalencia de pollos parrilleros seropositivos contra el virus de la laringotraqueítis infecciosa en Uruguay, mediante el uso de un "software" de inferencia Bayesiana que aplica la metodología de Monte Carlo basado en Cadenas de Markov (MCMC). En la investigación se incluyeron diecisiete granjas entre los años 2008 y 2009. Cada galpón fue seleccionado al azar en granjas ubicadas en la ciudad capital, Montevideo, y en los Departamentos de Canelones y Lavalleja. El tamaño de muestra necesario fue determinado en base al poder estadístico del análisis, y las muestras de sangre recolectadas fueron analizadas utilizando un ELISA comercial para la detección de anticuerpos contra el patógeno mencionado anteriormente. La seroprevalencia total del virus fue estimada en 31.5% [95% intervalo de credibilidad Bayesiano (16.8–49.2%); N = 1790]. Debido a que ninguno de los individuos bajo estudio habían sido vacunado contra el virus previamente al muestreo, la mayoría de los resultados podrían ser atribuidos a la exposición natural al virus de campo y/o al virus vacunal de aves en galpones vecinos. Esto debería ser tenido en cuenta como un elemento adicional del análisis de riesgo en la adecuada selección de vacunas para prevenir el contagio del virus a la población de pollos en Uruguay.*

**PALABRAS CLAVE:** epidemiología, herpesvirus, aves de granja

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## INTRODUCTION

Infectious laryngotracheitis is an acute respiratory disease of poultry brought by Gallid herpesvirus 1, a member of the *Herpesviridae* family (1). It is distinguished by conjunctivitis, respiratory distress, increased mortality, and losses of productivity. Since there is no treatment for this disease, policies for the containment of it are normally based on stopping contact between the agent and the host through biosecurity, and/or by vaccination. Meanwhile, prevalence is a scale of animal disease frequency that concentrates on existing status rather than new events. Serological diagnostic tests are regularly used for seroprevalence studies and, preferably, true seroprevalence (TP) should be estimated from apparent seroprevalence or percentage of samples classified as test–positive with adjusting methods such as Bayesian inference (2). The objective of this study was to estimate the TP of seropositive individual broilers against infectious laryngotracheitis virus in Uruguay using a Bayesian inference software based on Markov chain Monte Carlo (MCMC) technique.

## MATERIALS AND METHODS

### *STUDY AREA*

Uruguay has a poultry population of 14 million, a poultry meat production of 45,000 tonnes per year and a poultry egg production of 43,600 tonnes per year (3). The south of the country including the capital city Montevideo and Canelones Department has the concentration of chicken population (about 90% of total), because of in-and-around the big market Montevideo (4).

### *SAMPLE COLLECTION*

Seventeen farms of broilers older than 35 days of age were investigated. Each study flock was randomly selected at different farms selected from the capital city Montevideo, Canelones and Lavalleja (east of Canelones) Departments. None of the chickens had been inoculated against infectious laryngotracheitis virus 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% (5). The sample size in each of the farms was proportionally assigned (1% each of the total number of chickens at study farms) by the attainable financial, human and material means. The field study was implemented from October 2008 to April 2009 inclusive, comprised data collection through questionnaire interviews for each farm selected, in combination with blood sample collections for each chicken (questionnaire results were not treated with hereinafter).

### *LABORATORY EXAMINATIONS*

Blood samples collected were used for diagnostic investigations. Sera were analysed using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antibody to infectious laryngotracheitis virus in chicken serum (ProFLOK® ILT Ab Test Kit, Synbiotics Corporation, San Diego, CA). Positive and negative controls were included for each series of samples analysed. Absorbance was read on an ELISA reader at 405 nm. For estimating TP, the ELISA test sensitivity and specificity were not included into consideration due to unavailability of their publicised information.

### *DATA ANALYSIS*

Data were collected and entered into a database using the Base in the OpenOffice.org software version 3.1.1 (Sun Microsystems, Santa Clara, CA, USA). A random effects hierarchical model described below was fitted using Bayesian methods which facilitate the estimation procedure for fitting complicated hierarchical models (6). The model was fitted at the first level of our model including an individual study–specific parameter. The number of test positive samples $r_i$ for individual farm were modelled as a binary response variable with TP probability $p_i$:

$$r_i \sim \text{Binomial}\ (p_i,\ n_i)$$

At the second level of the hierarchy, the model was to assume that percentages of test positive were alike in some way. This was equal to specifying a random effects model for the TP probability $p_i$ as follows. They were assumed to be drawn from a common Normal population distribution:

$$\text{logit}\ (p_i) = b_i$$

$$b_i \sim \text{Normal}\ (\mu,\ \tau)$$

A standard non–informative prior is then specified for the population mean (logit) or probability of overall seroprevalence, $\mu$, with an alternative non–informative prior considered for the random effects variance (a uniform prior on the standard deviation), because of the absence of strong prior information:

$$\sigma \sim \text{Uniform}\ (0,\ 100)$$

$$\tau = 1\ /\ \sigma^2$$

The TP probability and associated 95%

Bayesian credible intervals (95% BCI) were computed via the Gibbs sampler, a MCMC technique, which was implemented using WinBUGS software (7). The exponential of these TP probabilities was taken to obtain overall seroprevalence estimates (Prev) and their 95% BCIs:

$$Prev = \exp(\mu) / (1 + \exp(\mu))$$

$$\mu \sim Normal(0.0, 1.0E{-}6)$$

Results presented here were based on multiple runs of length 100,000 following a burn–in of 10,000 iterations to achieve convergence.

## RESULTS

The 1790 broilers studied accounted for about 1.0 % of the study population and 0.01% of the total chicken population in Uruguay then. Blood samples collected from 1790 broilers in the study farms were serologically investigated. The statistical precision was slightly improved from ±2.5% to ±2.3% because of the eventual total number of samples of 1790 (larger than planned) and the overall percentage of test positive of 39% (95% CI: 37-42%, smaller than expected). All the farms had test-positive broilers (flock seroprevalence of 100% with 97.5% lower confidence limit of 80%). Percentages of test positive were highly variable between the study farms (1-90%) on the basis of different sample sizes (30-222). Table 1 shows the estimated TP against infectious laryngotracheitis virus among the study broilers categorised by farms. All the point estimates of TP were greater than 2%, between 2.6% and 88.8%. All the values for the percentage of test–positive were well within the Bayesian credible intervals of the estimated TP.

## DISCUSSION

When we think of determining the prevalence of any particular infectious diseases, several factors differ between studies, including study area, study period, sampling method and sample size. These variations between study designs make it difficult to draw generalisable conclusions regarding the prevalence of the diseases. Random effects hierarchical models strengthen the power of individual and relatively small studies by compiling results from independent studies (6). The strengthened power leads to a higher precision of the estimates, by that means decreasing the variance and more accurately pointing out notable results. Adjusted outcomes are required for precise comparison of seroprevalence estimates. One of the aims of the current study was to illustrate how a hierarchical modelling approach permits the dependable estimation of the uncertainty corresponding an individual broiler farm study's effect on outcome. The advantage of the approach taken in the study was that outcome data from all studies could be incorporated in one coherent inference framework, including relatively small samples. The hierarchical model pooled data across all field investigations to calculate the prevalence and Bayesian credible intervals thus making comparative assessment more robust, and more reliable (7). The overall antibody seroprevalence of infectious laryngotracheitis virus (31.5%) would be comparably high. Given the use of vaccine in the layer chicken population in Uruguay, most of these results could be ascribed to natural exposure by field viruses and/or vaccine viruses from neighbouring layers (8). It should be considered as further risk assessment for clarifying the suitable vaccines to prevent chicken population in Uruguay from the virus.

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