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Assessing the Seroprevalence Against Avian Pneumovirus and *Ornithobacterium rhinotracheale* in Broilers in Uruguay

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Abstract: The objective of this study was to estimate the true prevalence of individual chickens serologically test-positive against avian pneumovirus and *Ornithobacterium rhinotracheale* in Uruguay. Seventeen different broiler farms existed in three different provinces in Uruguay were recruited and the 1861 broilers were investigated. Individual-chicken sera were analyzed using a commercial enzyme-linked immunosorbent assay. The overall true seroprevalence was 1.9% [95% Bayesian Credible Interval (BCI): <1-7.4%] and less than 0.1% (95% BCI: 0-<0.1%) against avian pneumovirus and *Ornithobacterium rhinotracheale*, respectively. The result seroprevalence was relatively lower than that reported by other authors in a neighbouring country Argentina where the infection of these diseases was recently observed. This difference was discussed.

Key words: APV, bayesian model, ORT

INTRODUCTION

Respiratory diseases have historically been a major concern in commercial poultry production. A variety of pathogens has been identified as causing respiratory disease, acting either in a primary or secondary part. Avian pneumoviruses can lead to damage to the upper respiratory tract, such as, lack of cilia movement and/or cilia loss; damage that may lead to respiratory clinical signs such as coughing, sneezing, swollen head and more complexed respiratory troubles (Cook *et al*., 1988; Cook, 2000; Cook and Cavanagh, 2002; Gough, 2005). On the other hand, *Ornithobacterium rhinotracheale* has been connected with respiratory signs and growth retardation, in combination with increased mortality, fibrinopurulent pneumonia and airsaculitis. Increases in veterinary costs, increases in condemnation rate, drops in egg production, reduction of eggshell quality and decreased hatchability have been reported (Bisgaard *et al*., 2008; Van Empel *et al*., 2008). In Uruguay, seroprevalence of avian pneumovirus (Giossa *et al*., 2010) and *Ornithobacterium rhinotracheale* (Suzuki *et al*., 2010) infections at flock-level have been reported. To our knowledge, no report of seroprevalence of these infections at individual-chicken level in Uruguay has been publicized. The objective of this study was to estimate the True Prevalence (TP) of individual chickens serologically test-positive against avian pneumovirus and *Ornithobacterium rhinotracheale* in Uruguay, using Bayesian inference.

MATERIALS AND METHODS

Study area: Uruguay is located in the south-eastern part of South America, having a poultry population of 16 million, a poultry meat production of 76,000 tonnes per year and a poultry egg production of 53,500 tonnes per year (FAO, 2010). The south of the country including the capital city Montevideo and Canelones Department has the concentration of chicken population (about 90% of the total), because of in-and-around the big market Montevideo (Ministerio de Ganadería Agricultura y Pesca, 2010).

Sample collection: Seventeen farms of broilers aged older than 35 days were studied. Each study broiler was randomly selected at different farms selected from the capital city Montevideo, Canelones and Lavalleja (east of Canelones) Departments. None of the broilers had been inoculated against avian pneumovirus and *Ornithobacterium rhinotracheale* prior to sampling. The required sample size of 1537 in total from a chicken population of 16 million was sufficient to obtain a 95% confidence interval (95% CI) with a desired precision of ±2.5% when the estimated AP was 50% (Hintze, 2008). The sample size in each of the farms was proportionally assigned (1% each of the total number of broilers at study farms) by the attainable financial, human and material means. The field study was conducted between October 2008 and April 2009, comprised data collection through questionnaire interviews for each farm selected,

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together with blood sample collections for each broiler (questionnaire results were not treated with hereinafter).

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 and *Ornithobacterium rhinotracheale* (FlockChek® Avian Pneumovirus Antibody Test Kit and FlockChek® *Ornithobacterium rhinotracheale* 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 0.2 (titres larger than 396) and 0.4 (titres greater than 844) for avian pneumovirus and *Ornithobacterium rhinotracheale*, respectively were considered seropositive.

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). Seroprevalence estimates based on the use of an imperfect test, which is a nature of ELISA tests, must be corrected to take account of test performance. Based on the published ELISA specificity values [0.98 (= 602 samples test-negative out of 612 samples true-negative) and 1.00 (= 40 samples test-negative out of 40 samples true-negative)] for avian pneumovirus and *Ornithobacterium rhinotracheale*, respectively (IDEXX, 2003, 2004) and the expert's opinion to ELISA sensitivity for both the diseases (the most likely value for the sensitivity is 0.98 and 95% sure that the value exceeds 0.95) (IDEXX/Production Animal Services, 2009, Personal Communication), estimated TP of antibodies among study broilers at each farm were calculated. TPs for each farm were derived from the Apparent Prevalence (AP) using the Rogan-Gladen estimator (Rogan and Gladen, 1978) and information about the Sensitivity (Se) and Specificity (Sp):

$$TP = (AP + Sp - 1)/(Se + Sp - 1)$$

A Bayesian hierarchical model was used to derive posterior Bayesian estimates (denoted TP_B, Se_B and Sp_B, mentioned below) from prior distributions and the data from each broiler farm in this study. Consider estimation of the infection seroprevalence for a single farm where y broilers tested positive out of *n* broilers randomly selected. If the sample size (N) is much larger than n, then the sampling distribution of y is approximately binomial:

$$y|TP_B, Se_B, Sp_B \sim \text{Binomial}(n, AP)$$

Where TP_B is the true seroprevalence of infection in the samples and Se_B and Sp_B are the sensitivity and specificity, respectively, of the diagnostic test applied to each broiler sampled and $AP = TP_B*Se_B + (1 - TP_B)(1 - Sp_B)$. The authors modeled uncertainty about the Se_B and Sp_B of the diagnostic test using independent beta prior distributions (Vose, 2008):

$$Se_B \sim \text{Beta}(d + 1, n - d + 1)$$
$$Sp_B \sim \text{Beta}(d + 1, n - d + 1)$$

Where d is the number of desired (positive or negative) outcomes and n is the number of samples tested per farm. These values were decided by using the Se and Sp values for avian pneumovirus and *Ornithobacterium rhinotracheale* mentioned above. Especially for obtaining Se_B values for the two diseases, BetaBuster software (http://www.epi.ucdavis.edu/diagnostictests/betabuster.html) was used and the values "d + 1" and "n - d + 1" were produced as output. A beta distribution provides a flexible means of modeling uncertainty about parameters ranging between 0 and 1 (Baadsgaard and Jogensen, 2003).

At the second level of the hierarchy, the model was to assume that percentage of test positive were alike in some way. This was equal to specifying a random effects model for the true seroprevalence 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, μ, 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 true seroprevalence probability and associated 95% Bayesian Credible Intervals (BCIs) were computed via the Gibbs sampler, a Markov chain Monte Carlo (MCMC) technique, which was implemented using WinBUGS software (Lunn *et al*., 2000). The exponential of these

true seroprevalence probabilities was taken to obtain overall seroprevalence estimates (Prev) and their 95% BCIs:

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

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

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 1861 chickens investigated accounted for about 1% of the study chicken population and 0.01% of the total chicken population in Uruguay. All individual-chicken sera from the study area representing 17 farms were examined with the ELISA. Of all, 0.4% of the serum samples had test-positive against both avian pneumovirus and *Ornithobacterium rhinotracheale*. The proportion of all for test-negative against both the two diseases was 81%. Table 1 shows the estimated seroprevalence against avian pneumovirus among the study broilers categorized by farms. The numbers of broilers sampled between the 17 study farms were varied from 30-224. Thirteen out of the 17 farms had AP of greater than 0%, between 0.4 and 81%. All the point estimates of TP by Bayesian inference were greater than 0%. The APs of equal to 0% were adjusted greater by Bayesian inference. The Bayesian posterior sampling means for the SeB and SpB, estimated from the study, were 97.3% (95% BCI: 94.3-99.3%) and 98.7% (95% BCI: 97.8-99.4%), respectively and the overall true seroprevalence TP_B was 1.9% (95% BCI: <1-7.4%) (Table 2). Table 1 also shows the estimated seroprevalence against *Ornithobacterium rhinotracheale* among the study broilers. Thirteen out of the 17 farms had AP of greater than 0%, between 0.5 and 15%. All the point estimates of TP by Bayesian inference were greater than 0%. The APs of greater than 0% were adjusted lesser by Bayesian inference, while APs of equal to 0% were adjusted greater by Bayesian inference. The Bayesian posterior sampling means for the SeB and SpB, estimated from the study, were 97.4% (95% BCI: 94.4-99.3%) and 98.4% (95% BCI: 97.7-99.0%), respectively and the overall true seroprevalence TP_B was less than 0.1% (95% BCI: 0-<0.1%) (Table 2).

Table 1: Estimated seroprevalence against avian pneumovirus and *Ornithobacterium rhinotracheale* in broilers in Uruguay

		Avian pneumovirus (%)				*Ornithobacterium rhinotracheale* (%)			
				95% BCI				95% BCI	
Farm ID	n	AP	TP	Lower	Upper	AP	TP	Lower	Upper
1	91	81	83	74	91	1	<0.1	0	<0.1
2	89	0	0.3	<0.1	2	0	<0.1	0	<0.1
3	79	3	1	<0.1	5	15	14	7	23
4	97	3	1	<0.1	5	0	<0.1	0	<0.1
5	100	0	0.3	<0.1	2	1	<0.1	0	<0.1
6	100	27	26	18	36	2	<0.1	0	<0.1
7	100	4	2	<0.1	7	1	<0.1	0	<0.1
8	113	4	2	<0.1	6	0.9	<0.1	0	<0.1
9	111	7	6	1	12	5	0.2	0	3
10	119	6	4	<0.1	9	3	<0.1	0	0.5
11	200	5	3	<0.1	7	0.5	<0.1	0	<0.1
12	65	0	0.4	<0.1	2	0	<0.1	0	<0.1
13	224	0	0.1	<0.1	0.8	4	0.2	0	3
14	223	0.4	0.2	<0.1	1	0.9	<0.1	0	<0.1
15	30	10	7	<0.1	21	0	<0.1	0	<0.1
16	80	31	31	21	42	3	<0.1	0	<0.1
17	40	15	13	4	26	3	<0.1	0	<0.1

n; Number of chickens sampled, AP; Apparent Seroprevalence, TP; True Seroprevalence, 95% BCI; Bayesian Credible Interval

Table 2: Estimated seroprevalence against avian pneumovirus and *Ornithobacterium rhinotracheale* in broilers in Uruguay and its test characteristics

	5th percentile	Mean	95th percentile
Avian pneumovirus			
Overall true seroprevalence (TP_B)	<0.001	0.019	0.074
Sensitivity (Se_B)	0.943	0.973	0.993
Specificity (Sp_B)	0.978	0.987	0.994
Ornithobacterium rhinotracheale			
Overall true seroprevalence (TP_B)	0	<0.001	<0.001
Sensitivity (Se_B)	0.944	0.974	0.993
Specificity (Sp_B)	0.977	0.984	0.990

DISCUSSION

This study represents a moderate-scale seroepidemiological investigation on avian pneumovirus and *Ornithobacterium rhinotracheale* of broilers in Uruguay. The results of this study indicated that the seroprevalence of avian pneumovirus and *Ornithobacterium rhinotracheale* antibodies is relatively low in the study broilers in the area. The observed individual seroprevalence of the antibodies in this study (1.9% for avian pneumovirus and less than 0.1% for *Ornithobacterium rhinotracheale*) was lower than that reported by other authors in Argentina where the infection of these diseases was recently observed (Uriarte *et al*., 2010). However, several factors were different between studies, including study area, study period and sample size. These variations between study designs make it difficult to extract generalizable explanations with regard to the prevalence of any particular infectious diseases. Adjusted outcomes are required for accurate comparison of seroprevalence estimates. One of the aims of the present study was to illustrate how a hierarchical modeling approach permits the dependable estimation of the uncertainty corresponding an individual study's effect on outcome. The advantage of the approach used in the study was that outcome data from all studies could be incorporated in one coherent inference framework, including small samples. The hierarchical model data across all field studies to calculate the prevalence and BCIs thus making relative assessment more robust, and more reliable (Dohoo *et al*., 2003). The methodology was useful for obtaining estimates of avian pneumovirus and *Ornithobacterium rhinotracheale* prevalence and for establishing prevalence distributions which could be used as input parameters in risk assessment and decision models. The Bayesian stochastic approach is more complexed but relatively easily can be performed in the freely available software WinBUGS. Its advantage is that, in addition to providing posterior distributions for the TP_B, it also provides posterior distributions (estimates) for Se_B and Sp_B. However, knowledge and assumptions on the prior shape, value range and initializing values of the model inputs are needed.

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