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Stochastic Estimation of Seroprevalence Against *Ornithobacterium rhinotracheale* and Avian Pneumovirus among Chickens in Argentina

J. Uriarte, K. Suzuki, J. Origlia, D. Gornatti, M. Píscopo, R. Cerda, M. Herrero, H. Marcantoni, M.F. Unzaga, E. Spinsantti, F. Marino, M. Pecoraro, S. Corva and M. Petruccelli
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina

Abstract: The objective of this study was to estimate the true prevalence of seropositive individual chicken against *Ornithobacterium rhinotracheale* and avian pneumovirus in Argentina, using the Rogan-Gladen estimator in combination with Bayesian inference. Chicken runs existed in 21 and 20 different towns in Buenos Aires and Entre Ríos Provinces in Argentina for *Ornithobacterium rhinotracheale* and avian pneumovirus seroprevalence, respectively, were studied. Individual-chicken sera were analyzed using a commercial enzyme-linked immunosorbent assay. The 719 (for testing *Ornithobacterium rhinotracheale*) and 933 (for testing avian pneumovirus) chickens were investigated. The overall true seroprevalence was 62.6% [95% Bayesian Credible Interval (BCI): 37.6-84.5%] and 8.0% (95% BCI: 1.4-18.5%) against *Ornithobacterium rhinotracheale* and avian pneumovirus, respectively.

Key words: Bayesian inference, respiratory diseases, Rogan-Gladen estimator

INTRODUCTION

Prevalence is a scale of poultry disease frequency that concentrates on existing status rather than new events. Diagnostic tests are regularly used for prevalence studies and preferably, True Prevalence (TP) should be estimated from Apparent Prevalence (AP) by adjusting for test Sensitivity (Se) and Specificity (Sp) (Martin *et al*., 1987). In recent years, applications of Bayesian analytic methods (which are concerned with the consequences of modifying our previous beliefs as a result of receiving new data) for poultry-health prevalence survey data have increased. Use of Bayesian approaches gives a practical alternative for data analysis (Thrusfield, 2005; Vose, 2008).

Respiratory diseases have traditionally been a major concern in commercial poultry production. Various pathogens have been identified as causing respiratory disease, acting either in a primary or secondary role. *Ornithobacterium rhinotracheale* has been associated with respiratory signs and growth retardation, together with increased mortality, fibrinopurulent pneumonia and airsaculitis. Increases in medication 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). Meanwhile, avian pneumoviruses can cause damage to the upper respiratory tract (trachea), 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 complicated respiratory problems (Cook, 2000; Gough, 2005). In South America, serological evidence of the *Ornithobacterium rhinotracheale* (Arns *et al*., 1998) and avian pneumovirus (Peres *et al*., 2006) infections in Brazil have been observed. To our knowledge, no report of these infections in Argentina has been publicized. The objective of this study was to estimate the TP of seropositive individual chicken against *Ornithobacterium rhinotracheale* and avian pneumovirus in Argentina, using the Rogan-Gladen estimator in combination with Bayesian inference.

MATERIALS AND METHODS

Study area: Argentina is the eighth-largest country in the world by land area (the second largest country in South America), constituted as a federation of 23 provinces and an autonomous capital city, Buenos Aires. It borders Paraguay and Bolivia to the north, Brazil and Uruguay to the northeast and Chile to the west and south. Argentina has a poultry population of 604 million, a poultry meat production of 1.2 million tonnes per year and a poultry egg production of 480,000 tonnes per year (FAO, 2009). The centre of the country including Buenos Aires and Entre Ríos Provinces (north of Buenos Aires Province) had the concentration of chicken population (about 85% of the total), because of in-and-around the big market Buenos Aires (FAO, 2009).

Sample collection: Chicken runs existed in 21 and 20 different towns in the study area for *Ornithobacterium rhinotracheale* and avian pneumovirus seroprevalence, respectively, were studied. None of the chickens had been vaccinated against *Ornithobacterium rhinotracheale* and avian pneumovirus prior to sampling.

Corresponding Author: K. Suzuki, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina

The required total sample size of 601 from a chicken population of 513 million was sufficient to produce a 95% confidence interval (95% CI) with a desired precision of ±4% when the estimated AP was 50% (Hintze, 2008). The final sample size was determined by the accessible financial, human and material means. The field investigation was conducted between March 2007 and October 2008 for blood sample collections for each chicken.

Laboratory examinations: Blood samples collected were used for diagnostic investigations. Individual-chicken sera were analyzed using a commercial Enzyme-linked Immunosorbent Assay (ELISA) for the detection of antibody against *Ornithobacterium rhinotracheale* (FlockChek® *Ornithobacterium rhinotracheale* Antibody Test Kit, Dr Bommeli AG, a subsidiary of IDEXX Laboratories, Liebefeld-Bern, Switzerland) and avian pneumovirus (FlockChek® Avian Pneumovirus Antibody Test Kit, Dr Bommeli AG, a subsidiary of IDEXX Laboratories, Liebefeld-Bern, Switzerland), respectively. Positive and negative controls were included for each series of samples analyzed. 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 0.4 (titres greater than 844) for *Ornithobacterium rhinotracheale* and greater than 0.2 (titres larger than 396) for avian pneumovirus were considered seropositive, respectively.

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). Meta-analysis deals with the problem of reaching consensus on a particular question, using evidence from multiple independent studies (e.g. 21 *Ornithobacterium rhinotracheale* seroprevalence studies implemented in Argentina) (Sanchez *et al*., 2004; Dohoo *et al*., 2007). In this example, meta-analysis is based on the belief that the 21 studies that were actually done can be treated as a random sample from the population of studies. The purpose of each of these 21 studies was to estimate the overall true seroprevalence, each of the 21 studies had to draw samples randomly from its study population. Consequently, a meta-analysis is essentially a sample of samples. Each study population has a true (but unobserved) seroprevalence, which it estimated from a sample of subjects. 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 ELISA sensitivity values (mode of 98% with 95% certain that Se > 95% for *Ornithobacterium rhinotracheale*; mode of 98% with 95% certain that Se > 95% for avian pneumovirus) with the expert opinion and published ELISA specificity values (100% and 98.4%) for *Ornithobacterium rhinotracheale* and avian pneumovirus, respectively, estimated TP of antibodies among study chicken at each town were calculated (IDEXX, 2002, 2004). TPs for each town were derived from the AP using the Rogan-Gladen estimator (Rogan and Gladen, 1978) and information about the Se and Sp:

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

A hierarchical model, which is a type of meta-analyses mentioned above, consists of one layer of sampling above another. Two hierarchical models were made for monitoring infectious disease status or *Ornithobacterium rhinotracheale* and avian pneumovirus antibody seroprevalence in Argentina, respectively. Both models were fit using Bayesian methods which facilitate the estimation procedure for fitting complicated hierarchical models (Branscum *et al*., 2004; Papaspiliopoulos and Roberts, 2008). A Bayesian 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 town in this study. Consider estimation of the infection seroprevalence for a town where y chicken tested positive out of n chicken randomly selected. If the flock 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 flock and Se_B and Sp_B are the sensitivity and specificity, respectively, of the diagnostic test applied to each chicken 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 town. These values were decided by using the expert-specified Se values and published Sp values for *Ornithobacterium rhinotracheale* and avian pneumovirus mentioned above. A beta distribution provides a flexible means of modeling uncertainty about parameters ranging from 0-1 (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:

$$\text{Prev} = \exp(\mu)/(1 + \exp(\mu))$$

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

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 719 (for testing *Ornithobacterium rhinotracheale*) and 933 (for testing avian pneumovirus) chickens studied accounted for about $2*10^{-4}$% of the total chicken population in the study area. Table 1 shows the estimated seroprevalence against *Ornithobacterium rhinotracheale* and avian pneumovirus among the study chicken categorized by the study towns (n = 21 for *Ornithobacterium rhinotracheale*; n = 20 for avian pneumovirus). In the *Ornithobacterium rhinotracheale* study, the numbers of chicken sampled between the 21 study towns were varied from 3-180. Of all, 20 towns had AP of greater than 0%, between 20 and 100%. The AP of equal to 0% (Town G) was adjusted greater by Bayesian inference. All the point estimates of TP by Bayesian inference were greater than 0%. All the APs were well within the Bayesian credibility intervals, except for the town with AP of equal to 0% mentioned above. The Bayesian posterior sampling means for the Se_B and Sp_B, estimated from the study, were 97.7% (95% BCI: 95.1-99.3%) and 97.4% (95% BCI: 90.5-99.9%), respectively. The overall true seroprevalence TP_B was 62.6% (95% BCI: 37.6-84.5%). Meanwhile, in the avian pneumovirus study, the numbers of chicken sampled between the 20 study towns were varied from 3-194. Of all, 13 towns had AP of greater than 0%, between 2 and 44%. The APs of equal to 0% (Town D, E, G, I, L, Q and W) were adjusted greater by Bayesian inference. All the point estimates of TP by Bayesian inference were greater than 0%. All the APs were well within the Bayesian credibility intervals, except for the towns with AP of equal to 0% mentioned above. The Bayesian posterior sampling means for the Se_B and Sp_B, estimated from the study, were 97.3% (95% BCI: 94.3-99.3%) and 98.4% (95% BCI: 97.5-99.2%), respectively. The overall true seroprevalence TP_B was 8.0% (95% BCI: 1.4-18.5%).

DISCUSSION

This study represents the first moderate-scale seroepidemiological investigation on *Ornithobacterium rhinotracheale* and avian pneumovirus of chicken flocks in Argentina. The results of this study indicated that the seroprevalence of *Ornithobacterium rhinotracheale* and avian pneumovirus antibodies is relatively high in the flocks in the study area. However, several factors differed between studies, including study area, study period and sample size. These variations between study designs make it difficult to draw generalizable conclusions regarding the prevalence of any particular infectious diseases. Meta-analysis strengthens the power of individual and relatively small studies by compiling results from independent studies (Dohoo *et al*., 2003). 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 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 taken 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 investigations to calculate the prevalence and BCIs thus making comparative assessment more robust and more reliable (Lunn *et al*., 2000; Dohoo *et al*., 2003). The methodology was useful for obtaining estimates of *Ornithobacterium rhinotracheale* and avian pneumovirus prevalence and for establishing prevalence distributions which could be used as input parameters in risk assessment and decision models. The analyses provide some guidelines for use when interpreting *Ornithobacterium rhinotracheale* and avian pneumovirus prevalence results and when comparing results from studies using different study designs (study area and study period particularly).

Table 1: Estimated seroprevalence against *Ornithobacterium rhinotracheale* and avian pneumovirus among chickens in Buenos Aires y Entre Ríos Provinces in Argentina

	Ornithobacterium rhinotracheale					Avian pneumovirus				
				95% BCI (%)					95% BCI (%)	
Town ID	n	AP (%)	TP (%)	Lower	Upper	n	AP (%)	TP (%)	Lower	Upper
A	15	100	95	81	100	-	-	-	-	-
B	12	67	67	39	90	12	17	13	0.5	37
C	76	25	24	13	35	76	37	36	25	48
D	50	30	29	16	44	36	0	2	0.01	7
E	4	50	52	11	91	4	0	6	0.02	31
F	180	26	24	16	32	194	2	0.8	0.01	3
G	16	0	6	0.1	20	16	0	3	0.01	12
H	58	48	48	34	62	58	16	14	6	25
I	3	67	66	18	98	3	0	7	0.02	38
J	5	60	61	21	94	5	20	15	0.1	50
K	23	96	94	81	100	-	-	-	-	-
L	5	60	61	21	94	5	0	6	0.02	28
M	15	100	95	81	100	30	40	38	22	56
N	37	49	49	32	65	37	43	42	26	59
O	5	20	27	16	66	-	-	-	-	-
P	5	40	43	79	83	-	-	-	-	-
Q	34	35	35	19	53	34	0	2	0.01	7
R	43	88	90	78	98	86	14	13	6	21
S	43	68	66	50	80	43	26	24	12	38
T	57	60	60	46	73	-	-	-	-	-
U	33	97	96	87	100	18	28	25	8	47
V	-	-	-	-	-	7	43	36	7	71
W	-	-	-	-	-	20	0	2	0.01	11
X	-	-	-	-	-	138	44	44	35	53
Y	-	-	-	-	-	111	5	4	0.3	9

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

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