Flock-Level Seroprevalence Against *Ornithobacterium rhinotracheale* among Broilers in Uruguay

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Abstract: The objective of this study was to estimate the true prevalence of seropositive broiler flocks against *Ornithobacterium rhinotracheale* in Uruguay, South America. Seventeen farms of broiler chickens greater than 35 days of age were studied. The field investigation was conducted between October 2008 and April 2009. Individual-chicken sera and pooled sera (containing 10 individual-chicken sera each) were analyzed using a commercial ELISA for the detection of antibody against *Ornithobacterium rhinotracheale* in chicken serum. A total of 181 pooled samples from the study area representing 17 farms were examined. Fifty-four pools were classified as test positive, because they included at least one individual-chicken classified as positive. On the basis of deterministic approach, the estimates for the apparent prevalence and true prevalence at flock-level were 30% and 17%, respectively. The true prevalence estimate with the Bayesian model (stochastic approach) was slightly lower and having wider confidence intervals [11% (95% CI: 0%-32%)].

Key words: Bayesian inference, pooling, rogan-gladen estimator

INTRODUCTION

Respiratory diseases have usually been an important concern in poultry industry. Diverse pathogens have been recognized as causing respiratory diseases, acting either in a primary or secondary role. *Ornithobacterium rhinotracheale*, a lately reported pathogen, is a Gram-negative, pleomorphic, rod-shaped bacterium associated with respiratory disease, growth retardation, mortality and decreased egg production in poultry (Van Empel *et al.*, 2008). *Ornithobacterium rhinotracheale* can cause highly infectious diseases in poultry, but the severity of clinical symptoms, duration of the disease and mortality has been described to be highly variable (Bisgaard *et al.*, 2008). *Ornithobacterium rhinotracheale* can be a primary or secondary etiological agent depending on strain virulence, adverse environmental elements, immune condition of the flock, and presence of other contagious agents (Bisgaard *et al.*, 2008; Van Empel *et al.*, 2008). There are reports of *Ornithobacterium rhinotracheale* infections in Europe, Africa, Middle East, Asia, Far East and North America (Loock *et al.*, 2005; De Wit *et al.*, 2008; Farhoodi *et al.*, 2008; Van Empel *et al.*, 2008). In South America, serological evidence of the *Ornithobacterium rhinotracheale* infection in Brazil has been observed (Arns *et al.*, 1998). To our knowledge, no report of the *Ornithobacterium rhinotracheale* infection in Uruguay has been publicized.

Diagnostic tests are routinely utilized for poultry-health prevalence studies and ideally, True Prevalence (TP) should be estimated from Apparent Prevalence (AP) by modifying with test Sensitivity (Se) and Specificity (Sp). Absence of knowledge of or disregard for test errors (i.e. false positives and negatives) can lead to unsuitable sample size calculations for studies, misclassification of diseased and non-diseased conditions and prejudiced estimates of measures of result in risk factor studies. All of these opposingly influence disease studies, control and eradication programmes and, accordingly, animal trade. In recent years, applications of Bayesian analytic methods (which are concerned with the results of altering our previous beliefs as a result of adopting new data) for poultry-health prevalence survey data have increased (Herrero *et al.*, 2009; Origlia *et al.*, 2009; Suzuki *et al.*, 2009). The objective of this study was to estimate the true prevalence of seropositive broiler flocks against *Ornithobacterium rhinotracheale* in Uruguay using the Rogan-Gladen estimator in combination with Bayesian inference.

MATERIALS AND METHODS

Study area: Uruguay is located in the south-eastern part of South America bordering the South Atlantic Ocean, between Argentina in the west and Brazil in the northeast. Uruguay has a poultry population of 14 million, a poultry meat production of 45,000 tonnes per

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year and a poultry egg production of 43,600 tonnes per year (FAO, 2009). The south side of the country including the capital city Montevideo and Canelones Department had 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, 2009).

Sample collection: Seventeen farms of broiler chickens greater than 35 days of age were studied. Each study flock was randomly selected at different farms recruited from the capital city Montevideo, Canelones or Lavalleja (east of Canelones) Departments. None of the chickens had been vaccinated against *Ornithobacterium rhinotracheale* prior to sampling. The required total sample size of 1537 from a chicken population of 14 million was sufficient to produce 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 allocated (1% each of the total number of chickens at study farms) by the accessible financial, human and material means. The field investigation was conducted between October 2008 and April 2009, consisted of data collection through questionnaire interviews for each farm recruited, in conjunction with blood sample collections for each chicken (questionnaire results were not dealt with hereinafter).

Laboratory examinations: Blood samples collected were used for diagnostic investigations. Individual-chicken sera and pooled sera (containing 10 individual-chicken sera each) were analyzed using a commercial ELISA for the detection of antibody against *Ornithobacterium rhinotracheale* in chicken serum (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 series of samples analysed. For testing the pooled samples, the negative controls were not diluted at 1:10, which influenced the determination of a pool cut-off value. Absorbance was read on an ELISA reader at 650 nm. On the basis of the instruction manual of the ELISA kits, serum samples with sample to positive (S/P) ratios greater than 0.4 (titres greater than 844) were considered seropositive. For the flock-level validation, a pooled sample was classified as test positive if at least one individual serum sample included in the pool had S/P ratio greater than 0.4.

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). Each S/P ratio of all the pooled samples was used in a Receiver Operating Characteristic (ROC) curve analysis to derive a flock-level test classification. Within this analysis, the

optimal cut-off (S/P ratio) for a given pool to achieve maximum flock-level Se and Sp of the pool testing when compared to flock classification based on individual-chicken testing (used here as the gold-standard) was identified. As a descriptive measure of the ROC curve analysis, the Area Under the Curve (AUC) (that is maximum at 100% when both Se and Sp are 100%), was calculated using the Epi package version 1.1.7, in the R software version 2.9.2 (R Development Core Team, 2008; Carstensen *et al.*, 2009). The TP at flock-level was derived from the AP using the Rogan-Gladen estimator (Rogan and Gladen, 1978) and information about the Se and Sp:

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

For estimation of TP on the basis of deterministic approach (with 95% CI) above, Survey Toolbox software version 1.04 was used (Cameron, 1999).

A Bayesian model was used to derive posterior Bayesian estimates (denoted TP_B, Se_B and Sp_B) from prior distributions and the data from the study farm. Consider estimation of the seroprevalence where y chickens tested positive out of n chickens randomly selected. If the flock size (N) is much larger than n, then the sampling distribution of y is approximately binomial:

$$y \mid TP_B, Se_B, Sp_B \sim \text{Binomial} \left[n, TP_B * Se_B + (1 - TP_B)(1 - Sp_B) \right]$$

The authors modelled uncertainty about the Se_B and Sp_B of the diagnostic test using independent beta prior distributions (Vose, 2008):

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

Where d is the number of desired (positive or negative) outcomes and n is the number of samples tested. The infection seroprevalence using a mixture distribution was modelled:

$$TP_B \sim \text{Beta} \left(d + 1, n - d + 1 \right) \text{ with probability } \tau$$
$$TP_B = 0 \text{ with probability } 1 - \tau$$

Where d is the number of desired (positive or negative) outcomes, n is the number of samples tested and τ is the probability that the flock is infected. With this mixture distribution, computation of the posterior probability that the flock is not infected is possible and this computation can be performed easily using WinBUGS software version 1.4.3 under binomial-sampling schemes (Lunn *et al.*, 2000). A beta prior distribution can also be used for τ (Vose, 2008). Alternatively, τ can be set equal to an expert-elicited constant ($\tau 0$). The Markov chain-Monte Carlo simulation was run for 110,000 iterations of which

the first 10,000 iterations were discarded as 'burn-in'. On the basis of this stochastic approach, the posterior means and 95% CI (also called Bayesian credible interval") were recorded for the TP_u estimates and for posterior estimates of the test characteristics, Se_u and Sp_u.

RESULTS

The 1861 chickens studied accounted for about 1% of the study chicken population and 0.01% of the total chicken population in Uruguay. A total of 181 pooled samples (consisting of 10 individual-chicken sera each) from the study area representing 17 farms were examined with the ELISA. All individual-chicken samples in the pools were also examined with the same assay. Fifty-four pools were classified as test positive, because they included at least one individual-chicken classified as positive. In this study, no pools were considered as questionable based on the individual-chicken results. Figure 1 shows the ROC curve of flock-level screening test for seropositivity against *Ornithobacterium rhinotracheale*. A diagonal ROC curve (from lower left to upper right corner) indicates a diagnostic test which does not produce any useful differentiation between disease and non-diseased states. The ROC curve can be used to adjust cut-off values according to different diagnostic strategies as follows. If false-negatives and false-positives are equally undesirable, a cut-off on the ROC curve should be selected which is closest to the upper left corner of the X-Y chart. Based on the ROC curve analysis, a pool cut-off value for S/P ratio of 0.06 was determined. The AUC was 84%. At this cut-off value, the Se and Sp were estimated to be 80% and 80%, respectively (Table 1). The estimates for the AP and TP were 30% and 17%, respectively. The posterior Bayesian estimates for the TP_u, Se_u and Sp_u were 11%, 78% and 78%, respectively. Table 1 shows the estimated values for test sensitivity, specificity and true seroprevalence against *Ornithobacterium rhinotracheale* at flock-level including 95% CIs, on the basis of both deterministic and stochastic approaches.

DISCUSSION

This study represents the first moderate-scale seroepidemiological investigation on *Ornithobacterium rhinotracheale* in Uruguayan broiler flocks. The pool approach primary provided information on the seroprevalence of *Ornithobacterium rhinotracheale* at flock-level. Information on individual-chicken seroprevalence had to be estimated in a second step by assaying all sera in the positive pools. Depending on the objective of this study, this second step could be considered unneeded. However, determining flocks as positive on pool test results and the selected pool cut-off value would result in false-positive flock classifications, and would not provide the information on the within-flock seroprevalence. The authors therefore recommend
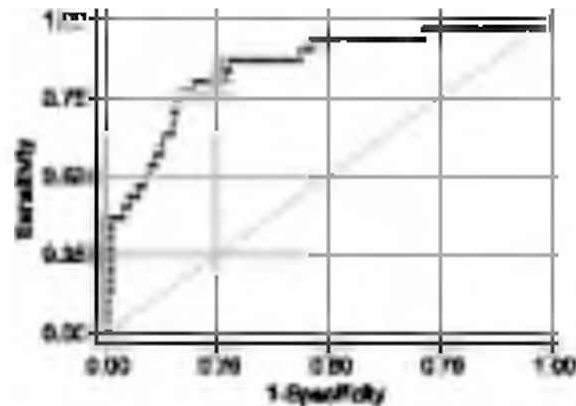

Fig. 1: Output Receiver Operating Characteristic (ROC) curve of flock-level screening test for seropositivity against *Ornithobacterium rhinotracheale* among broilers in Uruguay (n = 181)

Table 1: Estimated flock-level seroprevalence against *Ornithobacterium rhinotracheale* among broilers in Uruguay and its test characteristics (n = 181)

	5th Percentile	Mean	95th Percentile
Apparent seroprevalence	0.233	0.298	0.371
True seroprevalence			
Deterministic approach	0.123	0.166	0.208
Stochastic approach	0.000	0.112	0.319
Sensitivity			
Deterministic approach	0.614	0.800	0.923
Stochastic approach	0.613	0.775	0.901
Specificity			
Deterministic approach	0.729	0.801	0.862
Stochastic approach	0.701	0.779	0.851

investigating individual-chicken samples from positive pools whenever possible. The AUC is a popular measure of the accuracy of a diagnostic test. Other things being equal, the larger the AUC, the better the test is a predicted the existence of the disease. The AUC values greater than 90% indicate an extremely well-fitting model, values greater than 70% indicate a moderately well-fitting model and values approaching 50% indicating a model that is no improvement on random allocation of test status (Hintze, 2007). In this study, the AUC was 84%, which indicated a moderately well-fitting model. The ELISA used in this study satisfied the principal criteria (i.e. simpleness, speed, low cost, no specific equipment required and relatively high sensitivity and high specificity when assayed at the flock-level) needed for screening large numbers of samples in epidemiological studies. It nonetheless remains significant to modify the APs for the imperfect test characteristics. The authors used both a Rogan-Gladen estimator (deterministic approach) and Bayesian inference (stochastic approach). The approaches produced comparable TP estimates, with those of the

Bayesian model being slightly lower and having wider confidence intervals. The Rogan-Gladen estimator has the advantage that it is more-widely recognized and also can be utilized as a simple deterministic purpose (entering fixed values for AP, Se and Sp). One disadvantage is that estimator (for certain combinations of AP, Se and Sp) can return negative results. The Bayesian stochastic approach is more complicated but relatively easily can be conducted in the freely available software WinBUGS. Its advantage is that, in addition to supplying posterior distributions for the TP_B, it also supplies 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.

ACKNOWLEDGEMEMTS

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