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Flock-Level Seroprevalence against Avian Pneumovirus amongst Uruguayan Broiler Chickens

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Abstract: The objective of this study was to estimate the true prevalence of seropositive broiler chickens against avian pneumovirus at flock-level in Uruguay, using the Rogan-Gladen estimator in conjunction with Bayesian inference. A total of 181 pooled samples (consisting of 10 individual-chicken sera each) from the study area were examined with the enzyme-linked immunosorbent assay. All individual-chicken samples in the pools were also examined with the same assay. Forty-four pools were classified as test positive, because they included at least one individual-chicken classified as positive. The estimates for the deterministic (Rogan-Gladen approach) and stochastic (Bayesian approach) true prevalence were 30.9% [95% confidence interval (CI): 26.8-35.0%] and 31.4% (95% CI: 15.4-49.5%), respectively.

Key words: Bayesian model, poultry-health prevalence study, swollen head syndrome

INTRODUCTION

Respiratory diseases have generally been a significant concern in poultry industry. Various pathogens have been known as causing respiratory diseases, acting either in a primary or secondary role. Avian pneumovirus is well characterized as a pneumovirus in spite of the fact that it differs from mammalian pneumoviruses at the molecular level and has lately been categorized as the type strain of a new genus, *Metapneumovirus* (Gough, 2005). The illness caused by avian pneumovirus infection, called rhinotracheitis, was described at first in turkeys but shortly after in chickens also (Cook, 2000). In chickens, the role of avian pneumovirus as a primary pathogen is relatively unclear, although it is broadly recognized to be one of the elements involved in Swollen Head Syndrome or in another respiratory disease complex (Cook and Cavanagh, 2002). It is believed that avian pneumovirus can transmit chickens and bring about a specific antibody response, but not always associated with illness (Cook *et al.*, 1988). However, the virus has been isolated from chickens of various ages and chickens have been experimentally infected with avian pneumovirus (Cook, 2000; Gough, 2005). Shortly thereafter the first report of avian pneumovirus infection in South Africa and then France and the UK in the early 1980s, disease was described from other countries (Cook, 2000). Although most of the evidence is from serological studies rather than virus isolation, avian pneumovirus has now been reported throughout Europe and in Middle East, Far East, Central America and the USA (Gough, 2005). In South America, serological evidence of the avian pneumovirus infection in Brazil has been observed (Peres *et al.*, 2006). To our knowledge, no report of the avian pneumovirus infection in Uruguay has been publicized.

Diagnostic tests are usually used for poultry-health prevalence studies and, preferably, True Prevalence (TP) should be estimated from Apparent Prevalence (AP) by improving with test Sensitivity (Se) and Specificity (Sp). Absence of knowledge of or neglect for test errors (i.e. false positives and negatives) can cause inappropriate sample size calculations for studies, misclassification of diseased and non-diseased statuses and biased estimates of measures of result in risk factor studies. All of these opposingly affect disease studies, control and eradication programmes and consequently, animal trade. Currently, applications of Bayesian analytic methods (which are concerned with the results of improving our previous beliefs as a result of utilizing 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 avian pneumovirus 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

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million, a poultry meat production of 45,000 tonnes per year and a poultry egg production of 43,600 tonnes per year (FAO, 2009). 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, 2009).

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 avian pneumovirus 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 AP was 50% (Hintze, 2008). 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 were used for diagnostic tests. Individual-chicken sera and pooled sera (containing 10 individual-chicken sera each) were analyzed using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antibody against avian pneumovirus (FlockChek® Avian Pneumovirus Antibody Test Kit, Dr Bommeli AG, a subsidiary of IDEXX Laboratories, Liebefeld-Bern, Switzerland). Positive and negative controls were included for each assay. 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. 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) 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 larger than 0.2.

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 utilized 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 on the basis of individual-chicken testing (used here as the gold-standard) was determined. 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 estimated 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 based on 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 flock. 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:

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

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

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

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

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

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 carried out simply 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 (τ0). 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_B estimates and for posterior estimates of the test features, Se_B and Sp_B.

RESULTS

The 1861 chickens investigated 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 studied with the same assay. Forty-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 avian pneumovirus. 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 0.02 was determined. The AUC was 78%. At this cut-off value, the Se and Sp were estimated to be 61% and 92%, respectively (Table 1). The estimates for the AP and TP were 24% and 31%, respectively. The posterior Bayesian estimates for the TP_B, Se_B and Sp_B were 31%, 60% and 91%, respectively. Table 1 shows the estimated values for test sensitivity, specificity and true seroprevalence against avian pneumovirus at flock-level including 95% CIs, on the basis of both deterministic and stochastic approaches.

DISCUSSION

This study describes the first moderate-scale seroepidemiological study on avian pneumovirus in Uruguayan broiler flocks. The pool approach firstly provided information on the seroprevalence of avian pneumovirus at flock-level. Information on individual-chicken seroprevalence had to be investigated 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 unnecessary. However, determining flocks as positive on pool test results and the selected pool cut-off value would result in false-
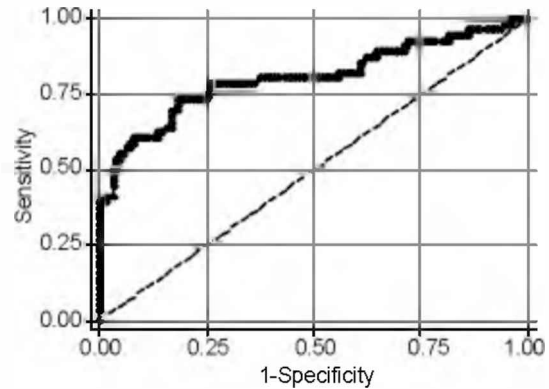

Fig. 1: Output Receiver Operating Characteristic (ROC) curve of flock-level screening test for seropositivity against avian pneumovirus among broilers in Uruguay (n = 181)

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

	5th percentile	Mean	95th percentile
Apparent seroprevalence	0.183	0.243	0.312
True seroprevalence			
Deterministic approach	0.268	0.309	0.350
Stochastic approach	0.154	0.314	0.495
Sensitivity			
Deterministic approach	0.468	0.607	0.735
Stochastic approach	0.466	0.595	0.717
Specificity			
Deterministic approach	0.858	0.920	0.961
Stochastic approach	0.855	0.912	0.955

positive flock classifications and would not give the information on the within-flock seroprevalence. The authors therefore suggest assaying individual-chicken sera 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 infection. The AUC values greater than 90% imply an extremely well-fitting model, values greater than 70% imply a moderately well-fitting model and values approaching 50% implying a model that is no improvement on random allocation of test status (Hintze, 2007). In this study, the AUC was 78%, 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 nevertheless remains important to improve the APs for the imperfect test characteristics. The authors used both a Rogan-Gladen estimator (deterministic approach) and Bayesian inference (stochastic approach). The approaches

generated comparable TP estimates, with those of the Bayesian model being slightly higher 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 sometimes return negative results. The Bayesian stochastic approach is more complicated but relatively easily can be implemented 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 required.

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