Stochastic Estimation of Optimal Age for Infectious Bursal Disease Vaccination in Broilers in Paraguay

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Summary

In Paraguay, vaccination programmes for infectious bursal disease (IBD) virus control planned by foreign vaccine manufacturers are commonly used, which are not always adapted to the particular conditions on the farm and to the status of maternally derived antibody (MDA) in chicks. The objectives of this study were to fit a generalised linear mixed model for MDA titre values for estimating optimal days of age for IBD vaccination in broiler flocks, and to assess how optimal vaccination timing estimates differ between flocks. The MDA titre values were measured by enzyme-linked immunosorbent assay (ELISA) with sera collected from 20 chicks per flock (n = 14) at 1, 8, 15 and 30 days of age. Markov chain-Monte Carlo method was used to fit a generalised linear mixed model for the dependent variable “log-transformed MDA” at 1, 8 and 15 days of age. Optimal days of age for IBD vaccination for the reference flock, and differences in optimal days of age between flocks were estimated. The study chicks were vaccinated according to the estimated optimal days of age. Data collected at 30 days of age were only used for checking a rise in antibody titres after vaccination. The mean log-transformed MDA titre values at hatch was estimated 12.35 [95% Bayesian credible interval (BCI) : 12.16–12.53] and half-life period of log-transformed MDA titre values was 3.7 days (95%BCI : 3.5–3.9). Given the use of intermediate vaccine with breakthrough titre value of 125, the optimal vaccination timing for the study flocks is at least seven days later than the recommended timing by the vaccine manufacturers. The results can be used as a standard to create IBD vaccination programmes, however it is recommended for estimating the vaccination timing to measure the MDA status on a routine basis.

Keywords : Gumboro disease, South America

Abbreviations :

BCI : Bayesian credible interval
ELISA : enzyme-linked immunosorbent assay
IBD : infectious bursal disease
IBDV : infectious bursal disease virus
lg : log scale
MCMC : Markov chain-Monte Carlo
MDA : maternally derived antibody

REML : restricted maximum-likelihood
VNT : virus neutralisation test

Introduction

Infectious bursal disease (IBD) virus (IBDV) is the etiological agent of “Gumboro disease” which is an important cause of economic losses in the poultry industry3. Antibody detection is a significant tool in the battle against IBDV. It is utilised to estimate the optimal timing for IBD vaccination when measuring the level of maternally derived antibody (MDA) in chicks. Vaccination in the presence of MDA titre values above the breakthrough titre of the vaccine is inefficient because this will cause neutralisation of the vaccine41.
The chicks should also not be left unprotected longer than necessary. It is therefore important that the optimal vaccination timing can be determined.

The MDA titre values measured by virus neutralisation test (VNT) have a constant rate of decrease with age\(^1\). This allows estimating the optimal vaccination timing on the basis of serology in newborn chicks. However, this very sensitive test is too expensive and time-consuming to be conducted on a regular basis. Meanwhile, enzyme-linked immunosorbent assay (ELISA) is comparatively inexpensive and results are acquired quickly and normally stable. In addition, titres of this assay may correlate well to VNT titres\(^2\). As a result, ELISA is often utilised for estimating optimal vaccination timing in the field\(^3\). Several calculation methods for estimating the optimal days of age for vaccination for a specific flock are created and used for routine application, based on the MDA titre values measured by ELISA, its variation, the genetic factor of the birds, and the IBD vaccine strain adopted\(^4^,\,5^\).

Previous studies have reviewed the age-based deterministic estimation method for optimal vaccination timing in chicks described above and made an effort to revise the method in conjunction with several different approaches, such as the possible use of sample-to-positive ratios \[
\frac{\text{sample mean} - \text{negative control mean}}{\text{positive control mean} - \text{negative control mean}}
\]
and the rate of weight gain in chicks\(^6^\). However, a statistical model connected with the concept of the age-based stochastic estimation method through inclusion of uncertainty in the model parameters has not been studied. The objectives of this study were to fit a generalised linear mixed model for MDA titre values for estimating optimal days of age for IBD vaccination in broiler chicks, and to assess how optimal vaccination timing estimates differ between flocks, using the field data in Paraguay.

Materials and Methods

Study area and flocks

The east side of Paraguay has the concentration of chicken population, especially in the surroundings of the capital city Asunción and the vicinity of the other urban areas such as Ciudad del Este in Alto Parana Department and Encarnación in Itapúa Department. Several live and killed vaccines against IBDV are imported in this country. Vaccination programmes with recommended timing for vaccination planned by foreign vaccine manufacturers are commonly used, which are not always adapted to the particular conditions on the poultry farm and to the status of MDA in chicks. A broiler chicken producers’ association covering the surrounding areas of Asunción provided access to a register of 48 members. Fourteen different farms were randomly recruited from the members of the association. Twenty broiler chicks in a flock were randomly selected from each of the 14 farms, resulting in a total of 280 chicks.

Field data collection

The examined 14 flocks of broiler chicks (Cobb breed) were derived from breeder flocks belonging to the same hatchery. They were monitored between March and July 2007. All the study poultry farms regularly used both a combination of “intermediate” and “strong” vaccine (in their pathogenicity) for IBDV control\(^12\), given at eight and 15 days of age, respectively, recommended by the foreign vaccine manufacturers (specific products’ name not shown). There had been no clinical symptoms of an IBDV infection for at least a year on these farms. Sera were collected from 20 chicks per flock at 1, 8, 15 and 30 days of age, and assayed by a commercial ELISA (FlockChek IBD, IDEXX Laboratories Inc., Westbrook, ME, USA) as indicated by the manufacturer instruction. None of the chicks had been vaccinated against IBDV prior to sample collection at 1, 8 and 15 days of age. After that, the chicks in each study flock were administered the intermediate vaccine according to the estimated optimal days of age described the following part “Model development”. Data collected were entered into a database using the Base in the OpenOffice.org software version 2.4.1 (Sun Microsystems, Santa Clara, CA, USA). The MDA titre values measured by ELISA values (the MDA titre values) were logarithmically transformed (log; scale) as “\(\text{lg (the MDA titre values)}\)”. The log-transformed data were checked for normality and turned out normal.

Model development

Based on the field data, the following stochastically-processed procedure was used to fit a generalised linear mixed model for the dependent variable “\(\text{lg (the MDA titre values)}\)”, to determine the slope and intercept of the curve and differences between the study flocks for the estimated optimal vaccination timing. Data collected at 1, 8 and 15 days of age were used in the model development. Data collected at 30 days of age were used for checking a rise in antibody titres after vaccination, and not included in the model development. The stochastic process is a random process in probability theory. The Markov chain-Monte Carlo (MCMC) estimates of parameters were obtained for the generalised linear mixed model including fixed effects “Slope”, “Intercept” and “Flocks”, and a random effect “Flocks” to demonstrate the dependence structure in the data. The Markov chain is a type of stochastic process where given what we can measure at present, the future is independent of the past. For the fixed effect “Flocks”, a flock which had the lowest coefficient was used as reference term. The result values of the restricted maximum-likelihood (REML) estimates of parameters for the generalised linear mixed
model with the same structure of data and coefficients described above were loaded as initial values (data not shown) for creating a simulation chain. The stochastic hierarchical model at $j$ days of age (corresponding “Days,” in the formula 1 mentioned below) was of the structure:

1. $Y_{i,j,k} \sim \text{Normal}(\text{Intercept}_i + \text{Slope}_i \times \text{Days}_j + \text{Flocks}_k + \text{Chicks}_k, \tau_j)$
2. $\text{Chicks}_k \sim \text{Normal}(0, \phi)$
3. $\tau_j = \tau_1 + \phi$

where $Y_{i,j,k}$ was the log-transformed MDA titre value of the $i$th chick, measured at $j$ days of age, in flock $k$. Non-informative priors were specified for the Intercept, Slope and Flocks; they were normal-distributed priors with mean = 0, and precision, the inverse of variance, $1 \times 10^{-9}, 1 \times 10^{-2}$, and $1 \times 10^{-3}$, respectively. Non-informative gamma priors of the $\tau_j$ and $\phi$ were also specified for the mean = 1, and precision $= 1 \times 10^{-2}$, and $1 \times 10^{-3}$, respectively. The MCMC simulation was run for 11,000 iterations of which the first 1000 iterations were discarded as ’burn-in’. The model was run in the WinBUGS software version 1.4.3\textsuperscript{6}. Missing log-transformed MDA titre values in the dataset were automatically replaced with plausible values generated by the built-in mis-singness at random function in the WinBUGS software, by monitoring the relevant $Y_{i,j,k}$ and the values generated were used in the analysis.

The mean log-transformed MDA titre values at hatch (0 day of age) and half-life period of log-transformed MDA titre values were estimated as follows:

$$\text{Mean log-transformed MDA titre values at hatch} = \frac{\sum_{k=1}^{f} (\text{Intercept}_k - \text{Slope})}{14}$$

$$\text{Half-life period of log-transformed MDA titre values} = (\text{Slope})^{-1}$$

where Intercept$_i$ was the estimated intercept in any flock $k$; and $f$ was the number of flocks ($n = 14$). The posterior means and 95% Bayesian credible interval (BCI) were recorded for each estimate. Kernel density plots of the mean MDA titre values at hatch and half-life period of MDA titre values were drawn, respectively.

Assuming the use of vaccines for IBDV control with breakthrough titre values (x) from 500 (assuming a strong vaccine used in the study farms) to 125 (assuming an intermediate vaccine also used there) at the study poultry farms\textsuperscript{3}, optimal days of age for IBD vaccination for the reference flock with the lowest coefficient in the model were estimated as follows:

Optimal days of age = $\frac{(\text{Intercept} - \text{lg}(x))}{(-\text{Slope})}$

The relationship between breakthrough titre values of vaccine and estimated optimal days of age was graphed, using the result values of the MCMC estimates. Graphics were produced using the R software version 2.7.1\textsuperscript{3}.

Differences in estimated days of age for IBD vaccination between the reference flock and other 13 flocks were compared on the basis of the unique value of the fixed effect “Flocks” for each of the study flocks. Vaccination timing recommended by vaccine manufacturers mentioned above was compared with the stochastic results using the model described above.

**Results**

Table 1 represents the structure of the data and descriptive statistics of dependent variable “lg (the MDA titre values)”. The missing data at the three time points accounted for 1.9% for the dependent variable. The log-transformed MDA titre values were variable between the study chicks, and temporal downward trends of those values for each flock were observed up to the 15 days of age. Due to logistic reasons, data for Flocks I to N at 30 days of age were not obtained. The log-transformed MDA titre values for Flocks A to H at days of age were not obtained. The log-transformed MDA titre value of the reference Flock A (Figure). Diﬀerences in estimated days of age for IBD vaccination.

**Discussion**

The MDA titre value obtained by ELISA is useful in estimating IBD optimal vaccination timing. The sera from newborn broiler chicks are examined by ELISA to estimate the time point for vaccination (expressed as days of age of the chicks in this study), using the software with age-based estimation method provided by the manufacturer of the
Table 1 Structure of the data and descriptive statistics of dependent variables [log, scale transformed maternally derived antibody; lg (the MDA value)] used in the analysis of estimation of the optimal age for IBD vaccination in broilers in Paraguay in 2007

<table>
<thead>
<tr>
<th>Flock</th>
<th>Number of samples</th>
<th>lg (the MDA value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 8</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>A</td>
<td>19.0</td>
<td>0.4</td>
</tr>
<tr>
<td>B</td>
<td>20.0</td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>20.0</td>
<td>0.7</td>
</tr>
<tr>
<td>D</td>
<td>20.0</td>
<td>0.8</td>
</tr>
<tr>
<td>E</td>
<td>20.0</td>
<td>0.6</td>
</tr>
<tr>
<td>F</td>
<td>20.0</td>
<td>0.5</td>
</tr>
<tr>
<td>G</td>
<td>20.0</td>
<td>0.5</td>
</tr>
<tr>
<td>H</td>
<td>20.0</td>
<td>0.9</td>
</tr>
<tr>
<td>I</td>
<td>20.0</td>
<td>1.8</td>
</tr>
<tr>
<td>J</td>
<td>18.0</td>
<td>1.5</td>
</tr>
<tr>
<td>K</td>
<td>19.0</td>
<td>1.9</td>
</tr>
<tr>
<td>L</td>
<td>20.0</td>
<td>2.0</td>
</tr>
<tr>
<td>M</td>
<td>20.0</td>
<td>2.0</td>
</tr>
<tr>
<td>N</td>
<td>20.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>276</td>
<td>268</td>
</tr>
</tbody>
</table>

N/A: Not available due to logistic reasons.

Fig. 1 Posterior distribution drawn by kernel density plots of the mean log-transformed MDA value at hatch (top) and half-life period of log-transformed MDA value (bottom)

Fig. 2 Estimated mean optimal days of age for IBD vaccination of Flock A (reference flock), changed with the vaccine breakthrough titre values. Dashed curves correspond to the 95 percent Bayesian credible intervals around the mean optimal days of age
ELISA kit, as is common practice. The results of this deterministic approach, however, provide only with an indication, partially because of no inclusion of uncertainty in the estimation method.

The authors used MCMC estimation method as stochastic approach. The chicks in each study flock were vaccinated according to the stochastically estimated optimal days of age. The results of our estimation could be partly supported by the rise in antibody titres after vaccination. The REML estimation method, which was implemented to obtain parameter estimates loaded as initial values for MCMC estimation method, is widely recognised and also used for a generalised linear mixed model as in a statistical analysis package such as R which is freely available. One drawback is that this method usually excludes subjects with totally or partly missing data. This may result in bias in estimating population parameters, if there is typical missingness in subpopulations. The MCMC estimation method is more complex but relatively easily can be conducted in software WinBUGS which is also freely available. Its advantage is to provide posterior distributions (estimates) for the mean log-transformed MDA titre values at hatch and half-life period of log-transformed MDA titre values (Figure 1) as well as optimal vaccination timing corresponding to the arbitrary breakthrough titre value of vaccine described above (Figure 2), at the same time of yielding parameter estimates for the generalised linear mixed model for the dependent variable “lg (the MDA titre values)”. However, knowledge and assumptions on the prior distributions, value range and initial values of the model inputs are necessary for implementing stochastic process.

Missing data are a major source of bias. Missing data, however, are inevitable in routinely collected longitudinal data and reflect the quality of data collection. In the REML estimation method, 12 subjects were excluded due to missing data items. Twelve chicks account for approximately half a flock and measures should be taken to incorporate that data. In this study none of the missing data were excluded. At each iteration of the simulation, missing data were imputed to the additional source of uncertainty acknowledged in all estimates. Although the authors assume in this study, that those data were missing at random, the flexibility of the current approach is such that informative missing data may easily be modelled.

The authors coped with the slope as constant between flocks in the model. This was because the study broiler chicks were from the same source and should have the identical genetic potentiality as Cobb breed regarding half-life period of MDA which basically influences the slope estimates. However, it was reported that the higher MDA titre values declined more rapidly than the lower initial titres. Therefore it remains possible that epidemiological conditions of the study poultry farms and/or elements in relation to the chicks determine the slopes. It suggests the necessity for further study into the properties of these various parameters.

The results of the stochastic model can contribute to a better understanding of the MDA status against IBDV in broiler flocks in the study area, and be used as a standard to create IBD vaccination programmes. Mean optimal days of age for IBD vaccination of Flock A is estimated about 15 days of age, given the use of intermediate vaccine. This

<table>
<thead>
<tr>
<th>Flock</th>
<th>Days</th>
<th>95% Bayesian Credible Interval</th>
<th>Rank (in ascending order)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (reference)</td>
<td>2.1</td>
<td>-0.46 4.7</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>3.8</td>
<td>1.3 6.5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>2.6</td>
<td>0.05 5.2</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>4.3</td>
<td>1.7 6.9</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>4.2</td>
<td>1.6 6.8</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>5.5</td>
<td>3.0 8.2</td>
<td>10</td>
</tr>
<tr>
<td>G</td>
<td>4.1</td>
<td>1.5 6.7</td>
<td>6</td>
</tr>
<tr>
<td>H</td>
<td>8.5</td>
<td>6.0 11.2</td>
<td>14</td>
</tr>
<tr>
<td>I</td>
<td>7.8</td>
<td>5.2 10.4</td>
<td>13</td>
</tr>
<tr>
<td>J</td>
<td>7.6</td>
<td>4.9 10.2</td>
<td>12</td>
</tr>
<tr>
<td>K</td>
<td>2.3</td>
<td>-0.29 4.9</td>
<td>3</td>
</tr>
<tr>
<td>L</td>
<td>5.5</td>
<td>2.9 8.1</td>
<td>10</td>
</tr>
<tr>
<td>M</td>
<td>5.3</td>
<td>2.8 7.9</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2 Differences of estimated optimal days of age for IBD vaccination from reference flock A in broilers in Paraguay in 2007.
vaccination timing is later than the recommended timing at 8 days of age by foreign vaccine manufacturers. The optimal vaccination timing for the other 13 flocks is much later than the recommended timing. Continuing adoption of the recommended vaccination timing should be reconsidered because vaccination for the chicks must be done in a reasonable period of time. Vaccination at 8 days of age is inefficient as this will cause neutralisation of the intermediate vaccine. Mean optimal days of age for IBD vaccination of Flock A is also estimated about 8 days of age, given the use of strong vaccine. The recommended timing at 15 days of age by the manufacturers is later than this estimated timing. It is implied that vaccination at the recommended timing targets at chicks which still have higher MDA titre values.

The stochastic model developed for this analysis demonstrates the importance of incorporating uncertainty into analyses relating to optimal vaccination timing for IBD control. It is always possible to invest into the development of more complex models, but these will not necessarily be more valid, and indeed due to their complexity and mathematical algorithms involved may be more difficult to communicate, and thereby be met with significant scepticism by stakeholders. In order to estimate optimal vaccination timing for IBDV control, measuring the MDA titre values by routine ELISA tests is recommended.

Acknowledgments

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References

原 著

巴拉グアイのブロイラー鶏に対する伝染性ファブリキウス囊病
ワクチン至適投与時期の確率論的推定

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要旨

巴拉グアイ国における伝染性ファブリキウス囊病ウイルス対策に係るワクチン投与プログラムは、海外のワクチン製造業者より提供されたものをそのまま使用するのが一般的である。しかしながら、こうした投与プログラムは当該国における生産現場の実情にいかに適応するか、農業業者における母鶏由来の移行抗体価が、ワクチン投与時期の相違によってどのように影響されるかを推定するための検討が必要であった。本研究では、バラグアイ国のブロイラー鶏群に対する伝染性ファブリキウス囊病ワクチンの至適投与時期を推定するために、当該移行抗体価の線形混合モデルへの適性を検討し、複数の推定モデル群ごとのワクチン至適投与時期の相違を推定結果を比較検討することを目的とした。当該移行抗体価を、全14群、20群の投与群を対象とし、それぞれ1、7、15及び30日齢時に採取した血清を用いて、ELISA法により測定した。

移行抗体価の対数変数を目的変数とする線形混合モデルの適合性には、マルコフ連鎖モンテカルロ法を利用した確率論的推定を行った。これにより、参照群に対するワクチン至適投与時期及び他の群との相違が、それぞれを推定した。

孵化時における移行抗体価の対数値は平均12.35（95%ペイズ信頼区間：12.16–12.53）、移行抗体価の対数値についての半減期は3.7日（95%ペイズ信頼区間：3.5–3.9）であった。誤診検出を考慮している、ワクチンメーカーが推奨する抗体価125のワクチン至適投与と（8日令）よりも、本研究による推定至適投与日の方が最短でも約7日遅く、8日令での投与では残存する移行抗体によりワクチンが中和されてしまう十分な予防効果が得られない可能性が示唆された。本研究の結果は、研究対象地域における既存の伝染性ファブリキウス囊病ワクチン投与プログラムの改善に資する考えられる一方、各群間の当該時期の相違（最大でおよそ9日間の開き）を考慮すると、可能な限り鶏の入囲に際し移行抗体価を測定し、その都度ワクチン至適投与時期を推定することがより望ましいと考えられた。

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