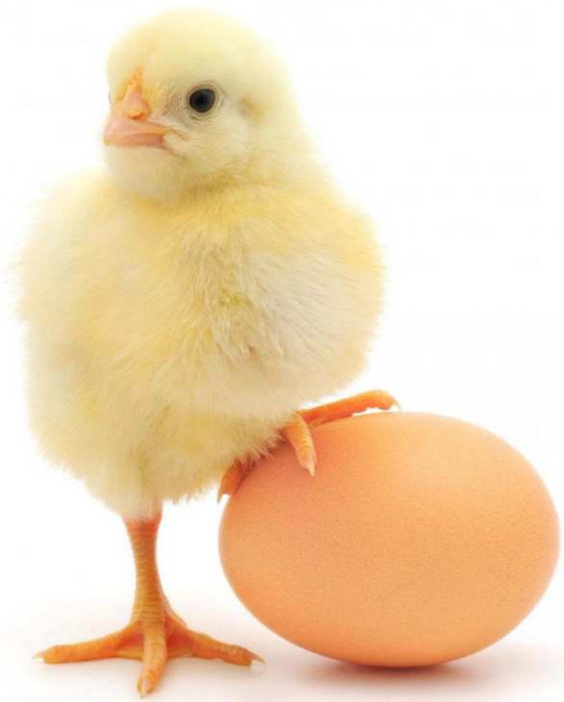
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 8 (4): 363-367, 2009
ISSN 1682-8356

The Use of Linear Mixed Models to Estimate Optimal Vaccination Timing for Infectious Bursal Disease in Broilers in Paraguay

K. Suzuki[1], J. Caballero[2], F. Álvarez[2], M. Faccioli[2], M. Goreti[2], M. Herrero[1] and M. Petruccelli[1]
[1]Laboratorio de Diagnóstico de Enfermedades de las Aves y los Pilíferos,
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata,
60 y 118, La Plata, B1900AVW, Argentina
[2]Laboratorio de Diagnóstico de las Enfermedades de las Aves, Facultad de Ciencias Veterinarias,
Universidad Nacional de Asunción, Ruta Mcal, Estigarribia N° 1085, Km. 11, San Lorenzo, Paraguay

Abstract: The objectives of this study were (1) to fit linear mixed models for Maternally Derived Antibody (MDA) values for estimating optimal days of age for Infectious Bursal Disease (IBD) vaccination in broiler chicks and (2) to evaluate how optimal vaccination timing estimates varied, based on the field data collected in Paraguay. The MDA titres were measured by Enzyme-linked Immunosorbent Assay (ELISA) with sera collected from 20 chicks per flock (n = 14) at 1, 8 and 15 days of age. Both Restricted Maximum-likelihood (REML) and Markov Chain-Monte Carlo (MCMC) estimation methods were used to fit linear mixed models for the dependent variable log-transformed MDA. There is a slight dissimilarity of each estimate between the two models because of the difference in mathematical algorithms and handling method of missing data. The study flocks with the earliest and latest estimated optimal timing for vaccination had a mean days of age of 15.1 [95% Bayesian Credible Interval (BCI): 13.3-17.0] and 23.7 [95% BCI: 21.7-25.7], respectively. This variation could be partly explained by a limited understanding of the true biological variability representing the variety of factors affecting MDA in the study chicks. Although the results of the study can be used as a benchmark to establish IBD vaccination programmes in the study area, it is recommended for estimating optimal IBD vaccination timing to measure the MDA level by ELISA tests on a routine basis.

Key words: ELISA, gumboro disease, modeling, South America

INTRODUCTION

Infectious Bursal Disease (IBD) Virus (IBDV) is the etiological agent of "Gumboro disease" named after the geographical location of the first recorded outbreaks in chickens in 1962 (Lukert and Saif, 2003). The IBD is an important cause of economic losses in the poultry industry. Antibody detection is a useful tool in the prevention against IBD. It is used to estimate the optimal vaccination timing when measuring the status of Maternally Derived Antibody (MDA) in chicks. Vaccination in the presence of MDA levels above the breakthrough titre of the vaccine is ineffective as this will lead to neutralization of the vaccine (de Wit *et al*., 2001). Meanwhile, the chicks should not be left insecurely longer than necessary. Therefore, it is crucial that the optimal vaccination timing can be determined.

The MDA titres measured by Virus Neutralization Test (VNT) have a steady decline rate with age (Berg and Meulemans, 1991). This enables estimating the optimal vaccination timing on the basis of serology in newborn chicks. This very sensitive test, however, is too time-consuming and expensive to be utilized on a routine basis. On the other hand, Enzyme-linked Immunosorbent Assay (ELISA) is rather inexpensive and results are obtained quickly and usually consistent. Moreover, titres of this assay may correspond well to VNT titres (De Herdt *et al*., 2005). Consequently, ELISA is often used for estimating optimal vaccination timing in the field (de Wit *et al*., 2001). Several formulas for estimating the optimal days of age for vaccination for a specific flock are used for routine application, based on the MDA level, its variation (coefficient value or CV), the genetic factor of the chicken, and the IBD vaccine strain used (Kouwenhoven and Van den Bos, 1994; de Wit, 2001).

Previous workers have reviewed the age-based estimation method for vaccination in chicks described above and attempted to revise the method in combination with several different approaches, such as the possible use of ELISA Sample-to-positive (S/P) values into MDA levels (De Herdt *et al*., 2005), the rate of weight gain in chicks (Vaziry *et al*., 2007) and reverse transcriptase-polymerase chain reaction technique (Block *et al*., 2007). However, a mathematical model closely connected with the concept of the age-based estimation method through inclusion of uncertainty in the model parameters, to the best of the authors' knowledge, has not been publicized. The objectives of this study were (1) to fit linear mixed models for MDA values to estimate optimal days of age for IBD vaccination in broiler chicks and (2) to evaluate how optimal vaccination timing estimates varied, based on the field data collected in Paraguay.

MATERIALS AND METHODS

Study area: Paraguay is an entirely landlocked country with a land area of 406,752 km^2, located in the centre of South America, bordering Argentina to the south and southwest, Brazil to the east and northeast and Bolivia to the northwest. The population is currently estimated at 6.2 million people, of which 3.6 million live in the capital city Asunción, its surroundings and other urban areas. About 40% of the total population engages in agricultural sector. The climate in Paraguay is subtropical with mean monthly temperature varying from 18°C in winter and 28°C in summer. Mean annual rainfall ranges between 750 mm and 1250 mm, increasing southwards. Paraguay has a poultry population of 17 million, a poultry meat production of 37,000 tonnes per year and a poultry egg production of 100,000 tonnes per year (FAO, 2008; FAO, 2001). In Paraguay, several live and killed vaccines against IBD are imported. Vaccination programmes with recommended timing for vaccination provided by foreign vaccine manufacturers are usually used, which are not always adapted to the specific conditions on the poultry farm and to the status of MDA in chicks.

Field observation: Fourteen flocks of broiler chicks from a commercial source were kept under observation. They were arranged between March and July 2007. All the study poultry farms regularly used an intermediate vaccine for IBDV control. Nothing has been reported on clinical symptoms of an IBDV infection for at least a year. Sera were collected from 20 chicks per flock at 1, 8 and 15 days of age and assayed by a commercial ELISA (FlockChek IBD, IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturer instruction. None of the chicks had been vaccinated against IBDV prior to sampling. 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). Data were checked for normality and the MDA values were logarithmically transformed (log_2 scale) as "lg (MDA)". Box-and-whisker plots of the log-transformed MDA values with the 14 flocks and three sampling points of time were drawn for visual assessment. Graphics were produced using the R software version 2.7.1 (Ihaka and Gentleman, 1996).

Modeling: Based on the field data, the following procedure was used to fit two linear mixed models for the dependent variable "lg (MDA)", to determine the slope and intercept of the curves and differences between the study flocks for the estimated optimal vaccination timing. Arguments "Day (1, 8 and 15, respectively)" were centred by subtracting the mean of 8, for producing an individual variable "Intercept" (= mean value of MDA at Day 8). The advantage of this was that the correlation between successive samples was reduced. Initially, Restricted Maximum-likelihood (REML) estimates of parameters were obtained for a linear mixed model including fixed effects "Slope", "Intercept" and "Flocks" and a random effect "Chicks" to represent the dependence structure in the data. For the fixed effect "Flocks", a flock with the lowest coefficient was used as reference term. The REML algorithm in nlme package in R was used in this analysis. Subjects with missing MDA values in the dataset were excluded automatically from the analysis. The statistical significance of the fixed effects was assessed and a P-value of less than 0.05 was considered statistically significant. The least-square means and 95% confidence interval (95% CI; so-called "Approximate 95% CI" in the nlme package) were recorded for each estimate.

Secondly, Markov Chain-Monte Carlo (MCMC) estimates of parameters were also obtained for a model with the same structure of data and coefficients described above. Appropriate prior distributions for each stochastic node were selected and the result values of the REML estimates were used as initial values for producing a simulation chain. The MCMC simulation was run for 11,000 iterations of which the first 1,000 iterations were discarded as 'burn-in'. The models were run in the WinBUGS software version 1.4.3 (Lunn *et al.*, 2000). Missing MDA values in the dataset were automatically replaced with plausible values generated by the built-in Missingness at Random (MAR) function in the software and the generated values were used in the analysis. The posterior means and 95% CI (sometimes so-called "Bayesian credible interval"or BCI) were recorded for each estimate.

Given the use of an intermediate vaccine for IBDV control with a breakthrough titre value of 125 (lg125≈6.97) at the study poultry farms, optimal days of age for IBD vaccination were estimated as follows:

$$\text{Optimal days of age} = (\text{Intercept}-6.97) / (-\text{Slope}) + 8$$

The relationship between estimated optimal days of age for IBD vaccination and proportion of the flock responding vaccination was graphed, using the result values of the MCMC estimates for the two flocks with the lowest and highest coefficients in the linear mixed model, respectively.

RESULTS

Field observation: Table 1 presents the structure of the data. The MDA data of 12 chicks were missed at least once. Data completely recorded at three sampling points of time of 268 out of 280 chicks were used for the REML estimates. On visual assessment using Fig. 1, the log-transformed MDA values of the study chicks were variable between individuals as well as flocks. Temporal downward trends of the MDA values for each flock were also observed.

Table 1: Structure of the data from 824 samples from broilers in Paraguay in 2007

Flock	No. of samples recorded			No. of samples sequentially recorded
	Day 1	Day 8	Day 15	
A	19	19	20	19
B	20	17	20	17
C	20	20	20	20
D	20	20	20	20
E	20	20	20	20
F	20	20	20	20
G	20	20	20	20
H	20	20	20	20
I	20	18	20	18
J	18	15	20	15
K	19	19	20	19
L	20	20	20	20
M	20	20	20	20
N	20	20	20	20
Total	276	268	280	268

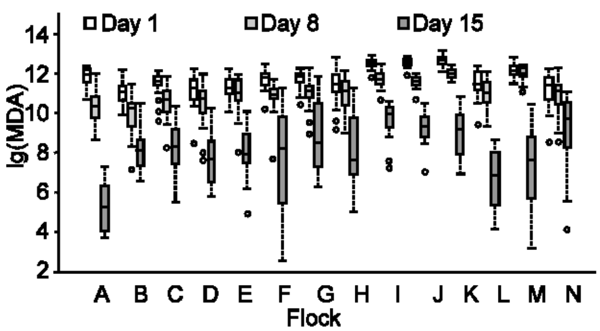


Fig. 1: Box and whisker plots of lg (MDA), with the 14 study flocks (A-N) and three sampling points of time (Day 1, 8 and 15), based on data from 824 samples from broilers in Paraguay in 2007

Modeling: In the first linear mixed model using REML algorithm, all the fixed effects had a $p<0.05$. The two final models for the dependent variable "lg (MDA)" are presented in Table 2. A line graph of the estimated optimal days of age for IBD vaccination (Fig. 2) was generated to evaluate how optimal vaccination timing estimates varied (1) according to proportion of the flock responding vaccination and (2) across flocks. With regard to the (1), Flock A and Flock I had an estimated mean optimal days of age for vaccination of 15.1 (95% BCI: 13.3-17.0) and 23.7 (95% BCI: 21.7-25.7), respectively. As for the (2), if one wishes to make 50% of the Flock A and Flock I respond the vaccination, the flocks should be vaccinated on about 16 and 24 days of age, respectively.

DISCUSSION

The MDA level obtained by ELISA is a practical tool in estimating IBD vaccination timing. The sera from newborn broiler chicks are assayed by ELISA to estimate the time point for vaccination (expressed as days of age of the chicks), using the software with age-based estimation method provided by the manufacturer of the ELISA kit, as is usual practice (De Herdt *et al.*, 2005). However, the results of this deterministic estimation are more likely to give only an indication (De Herdt *et al.*, 2005), partly due to no inclusion of uncertainty in the calculation.

The authors used both REML and MCMC estimation methods. The approaches yielded comparable parameter estimates, with those of the MCMC estimation method having narrower BCIs (Table 2). Strictly speaking, such quantities are different from parameter estimates and CIs in traditional statistical inference such as REML estimation method, but they are often interpreted in a similar way (Ihaka and Gentleman, 1996). The REML estimation method has the advantage that it is widely known and also can be used as a simple linear mixed model which requires a statistical analysis package such as R which is freely available. One disadvantage is that this method is not suitable to obtain an estimated optimal vaccination timing corresponding to an arbitrary proportion (e.g. 50% used in this study) of the flock to respond vaccination, in comparison with the latter estimation method. The MCMC estimation method is more complicated but comparatively easily can be implemented in software WinBUGS which is also freely available. Its advantage is to provide posterior distributions (estimates) for optimal vaccination timing corresponding to the arbitrary proportion described above (Fig. 2), at the same time of yielding parameter estimates for the linear mixed model for the dependent variable "lg (MDA)". However, knowledge and assumptions on the prior distributions, value range and initial values of the model inputs are required for conducting stochastic process (Lunn *et al.*, 2000).

A regular characteristic of many longitudinal studies is missing data among a subset of subjects. In this study the missing data was 1.9% for the selected variables. General techniques to cope with missing data are to exclude subjects with totally or partly missing data. However, this may result in bias in estimating population parameters, if there is distinctive missingness in subpopulations (von Hippel, 2004). The estimates of the parameters were quite consistent between two estimation methods (Table 2). There is a slight dissimilarity of each estimate between the two models because of the difference in mathematical algorithms and handling method of missing data. Discrepancies among the results derived from two different estimation procedures for the dependent variable suggest that multiple procedures should be considered when fitting such models.

The authors dealt with the slope as constant between flocks in each linear mixed model. This was because the study chicks were from the identical source and should have the same genetic potentiality regarding half-life period for MDA which basically has effect on the

Table 2: Growth curve models for Ig (MDA) using two different estimating algorithms, based on data from 824 samples from broilers in Paraguay in 2007

		Approximate 95% CI			95% BCI[c]	
Method	REML[a]	Lower	Upper	MCMC[b]	Lower	Upper
Fixed effects						
Slope	-0.274	-0.289	-0.258	-0.274	-0.290	-0.258
Intercept	8.90	8.39	9.42	8.91	8.42	9.40
Flock B	0.574	-0.152	1.30	0.577	-0.126	1.30
Flock C	1.05	0.332	1.77	1.05	0.343	1.76
Flock D	0.730	0.009	1.45	0.722	0.013	1.44
Flock E	1.18	0.455	1.90	1.17	0.468	1.89
Flock F	1.15	0.428	1.87	1.15	0.429	1.85
Flock G	1.51	0.792	2.23	1.52	0.816	2.23
Flock H	1.12	0.401	1.84	1.12	0.399	1.83
Flock I	2.34	1.62	3.07	2.33	1.63	3.04
Flock J	2.14	1.41	2.88	2.14	1.43	2.84
Flock K	2.08	1.36	2.81	2.08	1.36	2.78
Flock L	0.637	-0.084	1.36	0.630	-0.078	1.35
Flock M	1.50	0.778	2.22	1.50	0.800	2.21
Flock N	1.46	0.744	2.19	1.46	0.757	2.15
Random effects						
Chicks	0.756	0.538	1.06	0.729	0.490	1.00
Residual	1.71	1.51	1.93	1.73	1.53	1.95

[a]Restricted Maximum-likelihood (REML) estimates derived using nlme package in R. [b]Markov-chain Monte Carlo (MCMC) estimates derived using WinBUGS. [C]Bayesian credible interval.

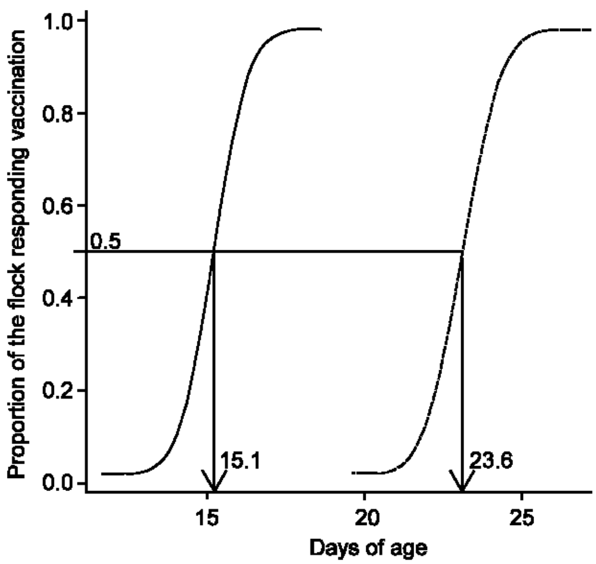


Fig. 2: Estimation of the optimal age for IBD vaccination based on the MDA level in broilers in Paraguay in 2007. Solid and dashed curves correspond to the Flock A (with the lowest coefficient in the linear mixed model) and I (with the highest coefficient in the model), respectively

slope estimates (de Wit, 2001). However, it remains possible that epidemiological circumstances of the study farms and/or factors related to the birds influence the slopes (De Herdt *et al*., 2005). It points out the need for further research into the properties of these various parameters.

Conclusion: In conclusion, the results of the study can contribute to a better understanding of the MDA status against IBDV in broiler flocks in the study area and be used as a benchmark to establish IBD vaccination programmes. Considerable caution is, however, required in interpreting these results and it does not necessarily follow that insisting on such programmes. There is more than one-week difference of estimated optimal days of age for vaccination between Flock A (the earliest) and Flock I (the latest). This variation could be partly explained by a limited understanding of the true biological variability representing the variety of factors affecting MDA in the chicks. In order to estimate optimal vaccination timing for IBDV control, measuring the MDA level by routine ELISA tests is recommended.

ACKNOWLEDGEMENT

This study was carried out as part of the project for the capacity development for improvement of livestock hygiene in the southern part of South America through regional cooperation [in Spanish: Proyecto de desarrollo profesional continuo para los veterinarios del Sur (PROVETSUR)], funded by the Japan International Cooperation Agency.

REFERENCES

Berg, T.P. and G. Meulemans, 1991. Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathol., 20: 409-421.

Block, H., K. Meyer-Block, D.E. Rebeski, H. Scharr, S. de Wit, K. Rohn and S. Rautenschlein, 2007. A field study on the significance of vaccination against Infectious Bursal Disease Virus (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV antibodies. Avian Pathol., 36: 401-409.

De Herdt, P., E. Jagt, G. Paul, S. Van Colen, R. Renard, C. Destrooper and G. Van den Bosch, 2005. Evaluation of the enzyme-linked immunosorbent assay for the detection of antibodies against Infectious Bursal Disease Virus (IBDV) and the estimation of the optimal age for IBDV vaccination in broilers. Avian Pathol., 34: 501-504.

de Wit, J.J., 2001. Gumboro Disease: Estimation of optimal time of vaccination by the Deventer formula. Proceedings of the 3rd meeting of working group 3 of COST action 839: passive protection and vaccination (current and future possibilities) in the presence of maternally derived antibody, Pulawy, pp: 21-28.

de Wit, J.J., J.F. Heijmans, D.R. Mekkes and A. Van Loon, 2001. Validation of five commercially available ELISAs for the detection of antibodies against infectious bursal disease virus (serotype 1). Avian Pathol., 30: 543-549.

FAO, 2001. FAOCLIM 2 (CD-ROM database): world-wide agroclimatic data, Rome.

FAO, 2008. FAOSTAT, Cited on 1 December 2008. Available from: http://faostat.fao.org/site/291/default.aspx.

Ihaka, R. and R. Gentleman, 1996. R: a language for data analysis and graphics. J. Comput. Graph. Stat., 5: 299-314.

Kouwenhoven, B. and J. Van den Bos, 1994. Control of very virulent infectious bursal disease (Gumboro Disease) in the Netherlands with more virulent vaccines. In: Kaleta, E.F. and U. Heffels-Redmann (Eds.), Proceedings of the international symposium on infectious bursal disease and chicken infectious anemia, Rauischholzhauzen, pp: 262-271.

Lukert, P.D. and Y.M. Saif, 2003. Infectious bursal disease. In: Saif, Y.M., H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald and D.E. Swayne (Eds.), Diseases of poultry, 11th Ed, Iowa State University Press, Ames, pp: 161-180.

Lunn, D.J., A. Thomas, N. Best and D. Spiegelhalter, 2000. WinBUGS-a Bayesian modelling framework: concepts, structure and extensibility. Stat. Comput., 10: 325-337.

Vaziry, A., D. Venne, D. Frenette, S. Gingras and A. Silin, 2007. Prediction of optimal vaccination timing for infectious bursal disease based on chick weight. Avian Dis., 51: 918-923.

Von Hippel, P.T., 2004. Biases in SPSS 12.0 missing value analysis, Am. Stat., 58: 160-164.