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Original Article

Application of Mathematical Models to Evaluate Serological Monitoring in Breeders and Commercial Laying Hens

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ABSTRACT

Mathematical models based on regression equations are an important tool for the interpretation of data generated by the serological monitoring of poultry flocks. The present study aimed to evaluate the serological monitoring of breeders and commercial laying hens by several poultry companies, and to develop mathematical models for seven different poultry diseases. Data from serological tests for seven diseases (chicken infectious anemia, infectious bronchitis, infectious bursal disease, Newcastle disease, avian encephalomyelitis, avian metapneumovirus, and avian reovirus) were selected for analysis in this study. The variables "age of birds at the time of blood collection" was considered as the independent variable (*x*), while "antibody titer" was set as the dependent variable (*y*). Analysis of variance and coefficient of multiple determination (R^2) were used to select the model with the highest capacity to adequately describe the analyzed data. Data from serological monitoring generated 166 linear and non-linear regression equations, but only 1.2% of them yielded an $R^2 \geq 0.8$ and more than 25 serum collections. The low number of suitable models may be related to the lack of standardization of the sample collection. In summary, serological monitoring of breeders and commercial laying hen flocks can be performed using mathematical models, but the lack of standardization of sample collections may have led to limited results.

INTRODUCTION

To achieve food safety standards, quality control must be met throughout the production chain (FAO, 2019). Quality control is based on decision criteria and a specific operational framework that help determine the final product quality, procedures which must be effective in generating good results at low cost (Feigenbaum, 1983). Reducing costs is a challenge because, in addition to the construction and preparation of poultry facilities and the selection of supplies to be used in feed production, an effective vaccination program must also be implemented.

Quality control programs must provide a periodic review of strategies and the use of statistical control methodologies, which could be associated with additional metrology and reliability techniques (Feigenbaum, 1983). However, the data generated through poultry health measurement systems are often subject to empirical assessment, which can be justified by the difficulty in interpreting the results of the serological monitoring of flocks. As such, the data produced by serological monitoring are partially lost or underused (Silva, 2000; Salle *et al.*, 2020).

To reduce health risks and financial costs, decisions associated with poultry production and health must meet the associated objective criteria (Salle *et al*., 2018). Previous studies conducted by our research group used artificial neural networks to evaluate and predict the productive performance of breeders, broilers, hatcheries, and slaughterhouses (Salle *et al*., 2001; Reali, 2004; Salle, 2005; Spohr, 2011; Tedeschi, 2019; Almeida *et al*., 2020; Oliveira *et al*., 2021). Furthermore, other studies used neural networks to predict the antimicrobial resistance of *Escherichia coli* strains of avian origin, and to evaluate lymphocyte depletion in the Bursa of Fabricius and thymus (Rocha, 2006; Moraes *et al*., 2010, Carvalho *et al*., 2016).

Mathematical models based on regression equations can aid in the interpretation of data generated by the serological monitoring of poultry flocks. These models allow the identification of patterns, explanation of variations, and prediction of future values (Pennstate, 2018). The establishment of interpretation and quality control criteria for serological results generated by poultry companies in southern Brazil using mathematical models was initially proposed by Salle *et al*. (1998, 1999). Both linear and non-linear regression equations have been proposed to describe the relationship between the animals' age and antibody titers presented by the birds for Newcastle, infectious bursal disease, and infectious bronchitis of chickens.

In this context, the present study aimed to evaluate the serological monitoring of breeders and commercial laying hens by several poultry companies, and to develop mathematical models for seven different poultry diseases.

MATERIALS AND METHODS

Serological data

Serological tests were performed using an enzyme-linked immunosorbent assay (ELISA) by two accredited laboratories linked to the Ministry of Agriculture and Livestock (MAPA) of Brazil (Brasil, 2013). Seven diseases were selected for analysis in this study: chicken infectious anemia, infectious bronchitis, infectious bursal disease (Gumboro disease), Newcastle disease, avian encephalomyelitis (AE), avian metapneumovirus, and avian reovirus. The methodology followed the protocols described by the manufacturers of the commercial kits. The results were expressed in the form of geometric mean titles (GMT).

Flocks

Data from 147,009 serum samples were collected from breeders and laying hens aged 4–66 weeks from 42 poultry companies in southern Brazil. Serological tests were conducted between 2017 and 2023.

Mathematical models

The variables "age of birds at the time of blood collection" and "antibody titer" were selected for the construction of the mathematical models. Moreover, the variable "age of birds" was considered as the independent variable (x), while "antibody titer" was set as the dependent variable (y).

Linear regression analysis was applied to adjust the simple linear, quadratic, and cubic models. Cubic order was established as the limit, because higher-order models are not reliable for making predictions or may produce predictions with a greater chance of error (Machado, 2006). Non-linear regression analysis was further applied to select the logistic model.

Statistical analysis

All analyses were performed using the JMP software, and a 95% confidence interval was adopted. A minimum of 25 serum collections were determined for each disease to establish a generalized model. Assessment of the homogeneity of variances indicated the need to transform the original data, with logarithmic transformation selected as it is normally related to the antibody titer (Salle *et al*., 1999). Logarithmic transformation is also commonly used to simultaneously stabilize the variance and solve the problems of heteroscedasticity and non-normality of the data (Salle *et al*., 1999; Kleinbaum *et al*., 2007).

Analysis of variance and coefficient of multiple determination (R^2) were used to select the model with the highest capacity to adequately describe the analyzed data. The R^2 value was calculated using the following equation:

$$R^2 = \frac{MSS}{TSS}$$

Where MSS represents the model Sum of Squares (the sum of the differences between the mean of the observed points and the points predicted by the model, squared), and TSS represents the Total Sum of Squares (the sum of the differences between the mean of the observed points and these points, squared).

RESULTS AND DISCUSSION

The periodic collection of serum samples is primarily used to monitor vaccine responses. The generated data also supports objective judgment and decision making for the optimization of flock health and production (Liebhart *et al*., 2023). Immunoassays based on the evaluation of humoral immunity can provide easy and rapid results at low cost (Priyanka *et al*., 2016). The use of humoral immunity as an interpretation criterion for serological results is based on the specificity of the antibody response induced by vaccination or viral infection (Abbas *et al*., 2021).

Considering its high flexibility, the quick turnaround, and the large sample sizes that require simultaneous processing, ELISA has been routinely used to monitor flock health, with commercial systems available for many poultry diseases (Liebhart *et al*., 2023). The miniaturization of ELISA to a 96-well microtiter plate further allows for a higher throughput of the assay and reduced costs (Makarewicz *et al*., 2015). The use of different substrates in ELISA has a major advantage, as the substrates bind to the respective conjugates specifically, subsequently developing coloration that can be read by an ELISA reader in terms of wavelength (Priyanka *et al*., 2016).

Adequate interpretation of ELISA results is an essential tool to improve vaccination programs and understand the actions required to improve them. Thus, monitoring serological responses is essential for the success of vaccination programs. Monitoring antibody titers further allows to determine the quality of the vaccine application method, and the field challenges caused by pathogenic agents. Furthermore, monitoring can be used to determine the relationships between antibody titers and production parameters (Salle *et al*., 2020).

The immune response and antibody curves were dynamic (TECSA, 2024). Many variables may interfere with the immunological response, including the prevalence of the disease, intercurrent immunosuppressive diseases, presence of maternal antibodies, local variant strains, genetic lineage of the animals, the environment where the birds are raised, and the quality of labor (Salle *et et al*., 2020). Furthermore, ELISA kits from different manufacturers may vary in terms of their specificity and/or sensitivity, resulting in conflicting titer profiles that must be considered when interpreting the results and supporting decision-making (Liebhart *et al*., 2023). As such, vaccination programs should be specific to each company, with respect to the characteristics of each region (Salle *et al*., 1999; Salle *et al*., 2020).

Differentiating between vaccine and pathogen challenge response antibodies is difficult, regardless of the clinical history of the flock. Currently, the interpretation criteria are subjective, and based only on the veterinarian's experience (Salle *et al*., 2020). As such, it is not possible to determine the expected immunological response in animals using traditional methods. Furthermore, serological tests detect only one immunoglobulin type, and may underestimate others; particularly immunoglobulin A, which is important for local immunity against viruses that penetrate the respiratory and intestinal tracts (Abbas *et al*., 2021).

Data from serological monitoring carried out using ELISA for seven poultry diseases generated 166 linear and non-linear regression equations (Supplementary Material). Of these models, only 1.2% had an $R^2 \geq 0.8$ and more than 25 collections. Two models with these characteristics were obtained, both for the AE in breeder flocks (Tables 1 and 2).

Table 1 – Regression equations obtained for avian encephalomyelitis (AE) in breeder flocks: Characteristics of generalizable models according to poultry fitness and age.

Samples (n)	Age (mean ± standard-deviation)[1]	GMT (log_{10})[2]	CV (%)[3]	R^2 (linear)[4]	R^2 (quadratic)[4]	R^2 (cubic)[4]
37	18.18 ± 3.52	3.358	26.73	0.469	0.781	0.845
69	24.04 ± 11.67	3.545	17.81	0.163	0.689	0.819

[1]*Age (weeks).* [2]*Geometric mean titer (GMT).* [3]*Coefficient of variation (CV).* [4]*Coefficient of determination (R^2).*

Table 2 – The adjusted regression equations according to the model obtained for avian encephalomyelitis (AE) in breeder flocks.

Model	Equation[1]	R^2 (cubic)[2]
Cubic	$y=4.0819906-0.0205628x-0.0223063(x-18.1892)^2+0.0032071(x-18.1892)^3$	0.8448
Cubic	$y=3.2171224+0.0256892x-0.0033643(x-24.0435)^2+7.19495(x-24.0435)^3$	0.8193

[1]*Geometric mean titer (GMT), log_{10} (y), and age [weeks] (x).* [2]*Coefficient of determination (R^2).*

The low number of suitable models obtained in this study may be related to the lack of standardization of the sample collection. There are currently no criteria establishing a specific age for birds, and sampling for monitoring is generally performed simultaneously with sampling for diagnosis. For example, Figure 1 presents the number of serum samples collections for serological monitoring of flocks vaccinated against avian metapneumovirus in one of the poultry companies evaluated in this study, sorted by bird age.

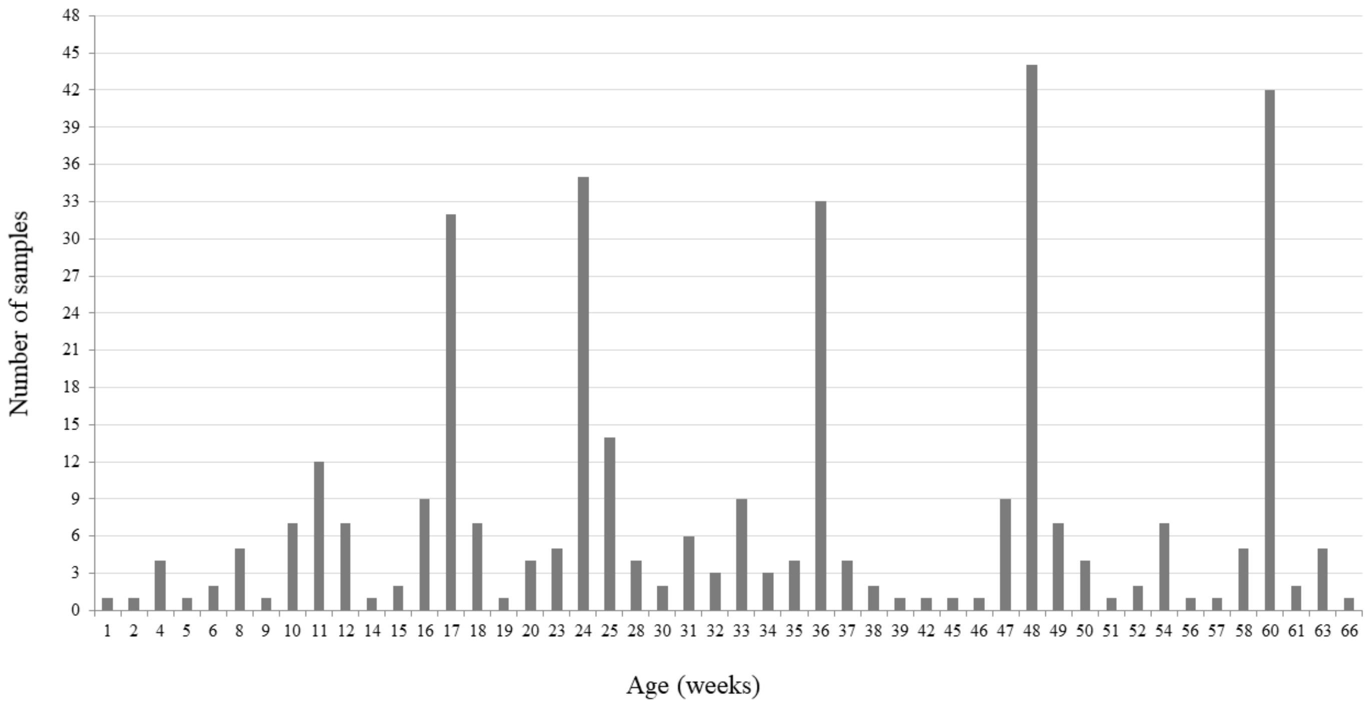

Figure 2 – Number of serum collections for serological monitoring of vaccination against avian metapneumovirus in one of the poultry companies evaluated in the study sorted by bird age.

In all cases, samples were collected at different ages in flocks with the same vaccination schedule, regardless of the disease and company evaluated. The lack of any standardization is reflected in the quality of the regression models generated for this poultry company (Table 3).

Table 3 – Regression equations for serological monitoring of vaccination against avian metapneumovirus in one of the poultry companies in this study.

Serum collection (n)	Age (mean ± standard-deviation)[1]	GMT (log_{10})[2]	CV (%)[3]	R^2 (linear)[4]	R^2 (quadratic)[4]	R^2 (cubic)[4]
356	34.45 ± 17.09	3.746	14.06	0.002	0.076	0.094

[1]*Age (weeks).* [2]*Geometric mean titer* (GMT)*.* [3]*Coefficient of variation* (CV)*.* [4]*Coefficient of determination* (R^2)*.*

The lack of standardization in the collection and interpretation of results is reflected in the higher cost of monitoring. It is worth noting that, depending on the target disease, monitoring may not be necessary until the end of the flock life, which must be considered when organizing collection protocols. According to the guidelines of the Brazilian Ministry of Agriculture and Livestock, breeder sampling collections for specific diseases, such as salmonellosis, can be performed in the middle of the rearing phase, at the beginning of egg production, and every three months thereafter (Brazil, 2016). However, for other protocols, the periodicity of collections may vary between once a month and every six weeks during the egg production phase (Herdt *et al.*, 2000). The costs of this monitoring include carrying out serological tests, the time spent on collection, possible zootechnical losses due to collection process, and the cost of logistics, packaging, and shipping of samples (Salle *et al.*, 2020).

Some procedures or errors related to sample collection are practical errors that can hinder the careful evaluation of results, such as successive thawing and freezing of samples, and the transfer of hemolyzed or contaminated samples (Jacobson, 1998; Newberry & Colling, 2021). Furthermore, a representative collection of the flock must be obtained, ensuring that a minimum number of 20 samples are collected, and that birds are selected randomly (Swets, 1988; Jacobson, 1998; Koerich, 2009); even though this error is less frequent in poultry farming. Serological monitoring is essential to guarantee the health of batches, but protocols must comply with this goal clearly and objectively, leaving no room for inconclusive interpretations (Silva, 2000).

The coefficient of variation (CV) is one of the parameters used to evaluate the quality of the immune response to vaccination. This CV measures the uniformity of detected antibody titers, and is an indication of the effectiveness of a vaccination program. In general, CVs that are lower than 30% indicate good uniformity of immunization (TECSA, 2024). In the current study, 92.17% (153/166) of the equations presented CV within these values, regardless of the monitored disease. The uneven CVs observed may be caused by the incompetence of vaccinators, the presence of illnesses that could affect the immune system, infections caused by the field virus before vaccination, among other factors (Salle *et al*., 2020). CV was related to the antibody titers obtained. In general, titers above 1000, associated with a low CV, indicate good vaccination (TECSA, 2024).

The R^2 value represents the amount of variation in the result explained by the model in relation to the total amount of variation, or how much of the variation in the result could be predicted by the model. The closer the value is to 1, the greater the predictive capacity of the model. The selection of models with an $R^2 \geq 0.8$ was established in the current study (Salle *et al*., 2003). However, a high value of R^2 does not necessarily indicate that the equation adequately predicts the results. This parameter is influenced by the fit of the regression line to the collected data, and mainly by the distribution and number of data points (Pennstate, 2023).

Other measures, such as the mean, mode, and median, also have disadvantages. These can be strongly influenced by extreme values, as well as the need to eliminate some of the collected data (Field, 2021). Nevertheless, the mean was used in the ELISA kit manufacturers' own software to minimize the difficulty in understanding the results of serological monitoring. To better generalize the models, a minimum of 25 collections were conducted per company (Pennstate, 2023). Models were considered generalizable only if the data represented the population from which they were sampled. Furthermore, the small sample size of the serum collection had a greater impact on the accuracy of the model than obtaining a lower R^2 (Field, 2021).

CONCLUSION

Serological monitoring data allowed for the creation of 166 regression equations for the seven poultry diseases analyzed in this study. Serological monitoring of breeders and commercial laying hen flocks can be performed using mathematical models. However, the lack of standardization of sample collections may have had a negative impact on the number of models with desirable characteristics that were obtained.

AUTHOR CONTRIBUTIONS

Conceptualization, LGBA, KAB, TQF, DTR, CTPS, VPN and HLSM; methodology, LGBA, TBW, and TBW; software, LGBA and CTPS; validation, KAB, TQF, DTR, CTPS and HLSM; formal analysis, LGBA, CTPS and HLS; investigation, LGBA, TBW and GZC; data curation, CTPS and HLS; writing—original draft preparation, LGBA, KAB and TQF; writing—review and editing, KAB and TQF; visualization, CTPS, VPN and HLS; supervision, CTPS, VPN and HLS; project administration, CTPS and HLS. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data will be available upon request.

CONFLICT OF INTEREST

None.

APPENDIX

Supplementary material will be available upon request.

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