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Research Article
Comparison of Three Different Vaccination Protocols Against Avian Infectious Anemia with One and Two Vaccines in Breeders and their Impact on Progeny

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Abstract
Background and Objective: Avian Infectious Anemia is widely distributed around the world causing problems for both breeders and broilers. The transmission of this virus can be vertical or horizontal and is characterized by a clinical and subclinical presentation respectively. In order to prevent avian infectious anemia, specific titers are given to the breeder and then passed on to the chick. The purpose of this study was to examine the effectiveness of double vaccination in achieving higher and more uniform titers as well as decreasing the number of negative birds. **Materials and Methods:** The present study compared a traditional single-vaccine versus two other vaccination protocols using two doses in different combinations in three different flocks of Cobb×Cobb breeders. In this research serological evaluation was performed using the Idexx Avian Infectious Anemia Ab Test Laboratory kit. The breeder and the chick at birth was evaluated at different stages. Then they were compared utilizing various statistical methods according to the nature of the data. **Results:** Significant difference was found in the breeders at 20 weeks in favor of the protocols that used two doses and at 24 weeks in favor of the protocols that used one dose, however no difference was found in one-day-old chick. **Conclusion:** An additional vaccination against chicken anemia virus does not appear to be beneficial.

Key words: Anemia, breeders, chicken anemia virus, progeny, vaccination

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Avian Infectious Anemia is a viral disease that is widely distributed around the world affecting both breeders and broilers[1]. Chicken anemia virus has recently been included in the family of Anelloviridae and is the only member of the genus Gyrovirus[2]. To date, only one serotype has been described but small differences in the pathogenicity of the various isolates have been documented[1,3].

This pathology has only been reported affecting *Gallus gallus* and it can be transmitted vertically or horizontally. It is characterized by a clinical and subclinical presentation respectively as birds gain resistance with age[4,5]. The clinical presentation occurs mainly in the first three weeks of life causing a significant increase in mortality, anemia, muscle hemorrhages, prostration, growth delays, atrophy of bone marrow and other lymphoid organs. On the other hand, the subclinical presentation will not manifest such strong effects in older birds with a more developed immune system. Instead, a decrease in productivity will result from the affectation[5]. The magnitude of the problem will depend upon factors such as the pathogenicity of the strain, the presence and amount of maternal antibodies, the age of the flock, presence of concomitant problems, etc[3,6].

Avian Infectious Anemia is a part of the immunosuppressive processes along with other diseases such as Marek's disease, infectious bursitis, leucosis, reovirus, among others. Therefore, it plays an important role in the presentation of multifactorial diseases facilitating the action of other agents[3-5].

In order to prevent Avian Infectious Anemia, specific titers are given to the breeder and then passed on to the chick. Currently this is achieved through vaccination, however some companies choose to expose their farms to the virus naturally because of frequent outbreaks[1,7,8]. In the past, contaminated liter was purposely introduced to healthy flocks as a method of exposure to the virus. However, these practices have fallen into disuse because of the associated health risks and a lack of sufficient and homogeneous exposure[9-11]. A live attenuated vaccine is usually administered during the growth phase. However, it has long been suggested that double vaccination should be utilized in order to achieve higher and more uniform titers, as well as decrease the number of negative birds[12,13].

MATERIALS AND METHODS

Location: The test was carried out on three flocks of Cobb × Cobb broiler breeders under commercial production conditions in a single complex with different modules located in the province of Puntarenas, Costa Rica.

Groups: The birds were divided into three groups (different commercial flocks, independent of each other of 30 thousand birds each). Group 1 (control group) received the vaccination program with a single dose of vaccine against Avian Infectious Anemia. The other two groups called the Treatment 1 (T1) and Treatment 2 (T2) received two doses of vaccine in different combinations against Avian Infectious Anemia.

Vaccines and protocols used: For this study, the vaccines Thymovac® from Elanco Laboratory (strain Cux-1) and Circomune® from Ceva Laboratory (Del Ros strain) were used. Table 1 shows the vaccination programs utilized. Each vaccination was audited to ensure proper application of the product.

Health status: The study was conducted in an isolated complex from other poultry farms, with strict biosecurity measures and with a long-term serological history to ensure that the flocks were first exposed to the virus when they were vaccinated. During the study, the groups received clinical and zoo-technical follow-up to ensure that there was no change that could affect the results of the study.

Serology: For monitoring, Serological tests were performed to monitor the breeders at 20 and 26 weeks of age. Moreover, serology was performed again in the first progeny of these birds. It was carried out by means of the Enzyme-Linked Immunosorbent Assay (ELISA) technique using the Idexx Avian Infectious Anemia Ab Test Laboratory Kit (dilution 1:100) according to the manufacturer's guideline.

Table 1: Vaccination programme by group

Groups	10 weeks	Application method	16 weeks	Application method
Control	N/A	N/A	Thymovac®	Drinking water
T1	Thymovac®	Drinking water	Circomune®	Wing puncture
T2	Thymovac®	Drinking water	Thymovac®	Drinking water

N/A: Not applicable

Statistical analysis: Data were analyzed using the descriptive statistics, Kruskal Wallis, one-way ANOVA and Chi-square test. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 25.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 2 shows serological results of the breeders at 20 weeks of age, a significant difference was observed between the control group and the T1 and T×2 ($p<0.01$), which presented lower coefficients of variation (CV%). In this trial the data was not normally distributed so the Kruskal Wallis test was used for analysis.

Table 3 shows the serological results of the breeders at 26 weeks of age, a significant difference was observed between T1 and control ($p<0.05$) but not between T1 and T2 or between control and T2, in the same way the groups with double vaccination had lower CV (%). The data was not normally distributed so the Kruskal Wallis test was used for analysis.

Table 4 shows the serological behavior of chicks from mothers of 26 weeks. In this case, the data was normally distributed and showed the homogeneity of variances, therefore data was analyzed using one-way ANOVA. In this analysis, significant difference was not found ($p>0.05$) and both the means and the CV (%) were very close to each other.

In a final stage, although there was no difference between the means of each group, an analysis of independence of variables (Table 5) was performed to determine whether there was a difference in their distribution. For this, the titers were reclassified according to their location in the histogram obtained at the time of reading the ELISA as described by Lind *et al.*[14]. Thus, the titers located in groups 0 and 1 were classified as "low", those in groups 2 and 3 were classified as "intermediate" and those in group 4 were classified as "high", however, no statistically significant difference was observed in this analysis ($p>0.05$).

Table 2: Serological results of the breeder at 20 weeks

Groups	Control	T1	T2
Average	8115	12347	12041
Median	11052[a]	13725[b]	13809[b]
CV (%)	69.37	23.74	29.67
Sample size	31	30	30

$p<0.01$

Table 3: Detail of the serological data of each group in the breeder at 26 weeks of age

Groups	Control	T1	T2
Average	9705	13218	11729
Median	11658[a]	13779[b]	13063[ab]
CV (%)	51.55	15.49	31.24
Sample size	30	30	15

$p<0.05$

Table 4: Serological behavior of chicks from mothers of 26 weeks of age in the different groups

Groups	Control	T1	T2
Average	7533	9436	7346
CV (%)	53.30	46.75	55.28
Sample size	45	30	15

$p>0.05$

DISCUSSION

There is a strong correlation between the level of antibodies in birds and the environmental excretion of the virus because vertical transmission from breeders having sufficient immunological protection is unlikely[7,8,15]. Likewise, it has been described that minimum levels of antibodies may not be a guarantee to overcoming difficulties in the field[15]. Challenges with clinical presentation occur when seronegative birds become infected during lay[16]. During this period, the breeders do not show any symptom, so the issue goes unnoticed that can prolong the detection time of the prevailing issue[4]. Historically, it has been believed that vaccination between 16 and 20 weeks guarantees protection in the progeny, however, vertical transmission can still occur[13].

It is customary to carry out a serological monitoring prior to the start of production in order to ensure that the mothers have the proper immunity[1]. This evaluation is generally carried out using the ELISA technique since other techniques such as virus neutralization and immunofluorescence are impractical when large groups of samples have to be analyzed[5,10]. Therefore, it was decided to use ELISA as a monitoring method.

In this study, breeder titers were compared with a single vaccine protocol against two other protocols where two vaccines were used at 10 and 16 weeks, respectively. There are three types of vaccine strains on the market: Cux-1, Del Ross and Cav P4, each with different characteristics in terms of virulence, immunogenicity, method of application, etc[17]. This test was carried out only with the Cux-1 and Del Ross strains. Generally, the usage of vaccines is suggested between 10 and 18 weeks of life or at least 5 weeks before start of lay to avoid vertical transmission[5,17,18].

Table 5: Comparison by groups

Group	Lows	Intermediates	Highs
Control	6	20	19
T1	6	13	11
T2	1	10	4

$p>0.05$

The immune response after vaccination should always be evaluated by means of the mean titer, but also with the CV (%) that refers to the uniformity of the data. Therefore, preventing avian infectious anemia requires a good and uniform seroconversion with a high mean serological titer and a low CV (%)[19].

The groups with double vaccination showed a significant difference ($p<0.01$) in bleeding at 20 weeks of age, which also show lower CV (%). In relation to this, it should be taken into account that T1 and T2 received the first vaccine at 10 weeks and the booster at 16 weeks, while the control group only received a vaccine at 16 weeks and this bleeding took place only four weeks later. It should be considered that avian infectious anemia is a viral disease that spreads slowly horizontally, which is why, when there are outbreaks, vertical transmission can take nine weeks during which the entire flock develops neutralizing antibodies to prevent the transmission of the disease[16].

Regarding the bleeding at 26 weeks of age, the differences were shortened and a significant difference ($p<0.05$) was observed only between the control group and the T1 but not between the control and the T2, or between both treatments. Here too, the CV (%) of the groups with double vaccination were lower than the control.

It should be noted that for this period a greater time was allowed for the virus to spread, all the groups received vaccination at 16 weeks and sampling was done ten weeks after the vaccination. This extended time described that it may take one entire flock rearing duration to achieve neutralizing antibodies[16].

Schwefer *et al.*[19] reported that birds vaccinated with the less attenuated strain (Cux-1) and via drinking water presented better titers and a lower coefficient of variation than the group that received the parenteral strain. However, a serological evaluation was performed only four weeks after vaccination.

Several authors agree that the vaccine strain that is used in water and that is produced in chicken embryos (Cux-1) is less attenuated than those applied parenterally and with this application, it is practically attenuated. What is done is a controlled natural infection[10,17]. However, it is possible that this virus has been attenuated due to the large number of passages and manipulations that this strain has undergone over the years[20].

Malo[15] reported that not all flocks respond to vaccination in the same way serologically, because differences were observed among farms. They also mentioned that the application of the Thymovac® vaccine responds very slowly when it was applied through drinking water, achieving high and sufficient titers to prevent vertical transmission up to 7 or 8 weeks after vaccination. Circomune® vaccine (Del Ros strain) is an attenuated virus vaccine in cell culture, highly invasive and for parenteral use only. This type of vaccine produces excellent immunity through its route of application[17,21].

On the contrary, the use of less attenuated strains in drinking water can result in a lower immunogenic capacity, which may be linked to an interaction, for example, with maternal antibodies in birds[17,22]. Due to the large number of defense cells present in the digestive system, it could eventually have a similar effect when given via drinking water to birds with previously acquired immunity. On the other hand, the inconveniences associated with applying mass vaccines to water, as in this case and the impossibility to ensure a complete dose per bird must also be considered[23].

Schwefer *et al.*[19] reported that the speed of vaccine diffusion and the presence of birds with low antibody titers is due to the type of vaccine used under field conditions. Moreover, Chicken infectious anemia may be prevalent in day old chicks when the breeder flock has an average titer. Various authors have suggested that the virus can persist in the gonads of seropositive birds and therefore a small percentage of the progeny will contain viral DNA[2,6,16]. The importance of this latency in commercial production conditions is not entirely clear, however, it could play an important role in the sudden outbreaks, especially when the bird's immune system is not continuously stimulated by the contact with the field virus due to excessive cleaning[17].

No significant difference was found ($p>0.05$) in the analysis of the titers of the chicks from the first born of each group and the means and CV (%) were very close to each other. Despite the fact that the progeny of T1 had a higher average in the analysis of the data of the mothers at 26 weeks. It should be noted that the transfer of antibodies is not a passive and invariable process and it has been observed that mothers with high titers will not necessarily transfer them to their progeny. It may depend on various factors such as the condition of the mothers, the mating partner, sex of the progeny or even the position of the egg in the mother's laying series. However, many of these processes are still not fully understood[24].

In the past, various studies have been carried out to establish the minimum protective titer to prevent vertical transmission of avian infectious anemia. One of the first was

elaborated by Malo and Weingartenin[15] who determined that birds require at least a $\log_2$ 8 titer in virus neutralization to avoid the passage of the virus to their progeny. On the other hand, Idexx Laboratories indicates that in its ELISA kit (1:100 dilution) this value is equivalent to titers greater than 1000, which are located in group 1 of its histogram[25].

A comparative study between titers of each treatment was done, classifying them as low, medium and high as suggested by Munoz *et al*.[25] with the idea of evaluating protocols that generate greater number of titers. There was no significant difference in this comparison either ($p>0.05$).

CONCLUSION

According to the results of the present study, an additional vaccination against Chicken anemia virus does not appear to be beneficial. It is important to take into account the vaccination protocols and not all vaccines are registered in the various countries. Likewise, different vaccine strains differ in virulence and immunogenicity, therefore, the research should be repeated with other combinations, or compared between different vaccine strains. Due to the particular situation of each company, an analysis must be carried out to evaluate if the second dose is necessary and economically viable depending upon the circumstances.

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