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Use of Egg Yolk Antibodies to Predict Optimal Age of Vaccination Against Infectious Bursal Disease (IBD) in Broilers

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Abstract: Collecting sera from new-born chicks requires their sacrifice, which is an invasive and little economic method of sampling. We studied the possibility of replacing the antibody titres in serum of chicks by those in pre-incubated egg yolk for determining the optimal Age of Vaccination (OAV) against Infectious Bursal Disease (IBD) by the Deventer formula. The study focused on 10 hyper-immunized broiler-breeder flocks. Yolk of pre-incubated eggs and sera of newborn chicks, originated from the same parent flock, were essayed by Enzyme-Linked Immunosorbent Assay (ELISA) to detect IBDV specific Maternally Derived Antibodies (MDA). ELISA titres in both types of samples were similar ($p<0.05$) and well correlated ($r = 0.74$, $p<0.05$). The difference between the two vaccination age estimations, based on egg yolk and serum antibodies respectively, was minimal (less than one day). This difference was independent of parent breeder-flock age ($r = 0.04$, $p>0.05$ NS). These results encourage the use of egg yolk in predicting the OAV against IBD instead of sera from sacrificed chicks.

Key words: Vaccination age, IBD, egg yolk, serum, chicks, ELISA

INTRODUCTION

While the widespread use of recombinant vaccines (Haddad *et al*., 1997; Gagic *et al*., 1999; Goutebroze *et al*., 2003) and immune complex vaccines (Jeurissen *et al*., 1998), considered as insensitive to MDA, vaccination against IBD remains problematic in most countries. Indeed, the advent of hypervirulent virus had forced vaccinations at more and more early ages, thereby increasing the risk of vaccine failure following vaccine neutralization by MDA (Block *et al*., 2007), which can be present at high levels during the first days of chick's life.

So, we understand the importance of the vaccination at the time when the level of residual antibodies is low enough to allow a vaccine take but high enough to minimize the length of the immunity gap, during which, some birds still susceptible to the wild virus, but at the same time refractory to the vaccine one (van den Berg and Meulemans, 1991).

Notwithstanding its usefulness, the prediction of the optimal Age of Vaccination (OAV) against IBD is not a common practice and the chickens continue to be vaccinated at the timing provided by foreign vaccine manufacturers. The mathematical formulas used in predicting the OAV use serum antibodies detected by ELISA technique. This test is simple, quick, tests a large number of samples at the same time and is adaptive to automation to computer software (Lukert and Saif, 2009). In front of these advantages, sera are obtained after sacrificing chicks, which is a binding, expensive and invasive method of sampling.

Passive transmission of parental humoral immunity to the offspring via the egg yolk (Rose *et al*., 1974; Tressler and Roth, 1987; West *et al*., 2004) is reminiscent of the existence of a dependent relationship between antibody rates in hen's plasma, in egg yolk and in chick's plasma.

Many studies have demonstrated the good relationship of dependency between the rate of serum antibodies in breeders and that found in the egg yolk, they produce (Brown *et al*., 1989; Rauber *et al*., 2004; Jeong *et al*., 2010). Others found no significant difference between the rates of antibodies in both types of samples (Brown *et al*., 1991; De Wit *et al*., 2001). The intensity of this relationship would not be the same for all viral agents (Silim and Venne, 1989; Keck *et al*., 1993). Regarding IBDV, Silim and Venne (1989) have highlighted a very strong correlation ($r = 0.9$), which makes possible, the evaluation of the hyper-immunization process in breeders through the titration of the egg yolk IBDV specific antibodies.

If the relationship between the serum antibodies in breeders and egg yolk has been copiously studied, that of antibodies in serum chicks and in egg yolk has not attracted the same interest, no doubt due to the lack of applications.

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The purpose of this study was to predict the optimal age of IBD vaccination using ELISA MDA titres in the egg yolk instead of sera from sacrificed chicks without affecting the vaccination age estimation.

MATERIALS AND METHODS

Experimental design: Ten broiler-breeder flocks of different ages and well-known IBD vaccination history were selected for the needs of the experiment.
Twenty fertilized and pre-incubated eggs per flock were collected at the hatchery on the day of laying or two to three days later. After hatching, sera were collected from twenty sacrificed one-day-old chicks. Sera and egg yolk derived from the same parent flock were essayed by commercial ELISA kit to evaluate MDA titres for predicting optimal age of IBD vaccination using Deventer formula, an age-based estimation method.

Separation of egg yolk antibodies: The extraction method is that described by Al-Natour *et al.* (2004) with some slight modifications. The yolk was separated from the albumen using a commercial egg separator. Then, it was rolled on absorbent paper to remove all traces of egg white. Subsequently, the egg yolk membrane was broken and one milliliter of the yolk was placed in a polypropylene centrifuge tube and combined with 1.5 ml of Phosphate-Buffered Saline (PBS) and mixed thoroughly with a vortex shaker until a thick paste appeared. The mixture was allowed to set for 30 minutes at room temperature and then centrifuged at 2000xg for 20 min. Eventually, 0.25 ml of the upper aqueous clear layer was removed and diluted in 0.25 ml of PBS. The egg yolk, then pre-diluted 1:5, was kept at 4°C for testing.

Extraction of sera: Blood was collected in dry plastic tubes of 5 ml. The tubes were kept upright for 2 to 3 hours at room temperature until complete coagulation of blood. Clotted blood in the tubes underwent centrifugation at 2000xg for 30 min at 4°C. Sera were collected in aliquots and stored at -20°C until performing ELISA test.

Titration of IBDV specific maternally derived antibodies: The level of IBDV specific antibodies in both types of samples (sera and egg yolk) were detected by a commercial ELISA kit: IDEXX Flock Chek IBD Standard (batch number: 09260-ME557), one of the recommended commercial kits for taking advantage of the Deventer formula.

Evaluation of the uniformity of MDA titres: The uniformity of MDA titres was précised by the coefficient of variation (CV = standard deviation/mean).

Vaccination age estimations: The optimal ages of IBD vaccination based on serum titres or egg yolk titres were determined by the Deventer formula (De Wit, 2001):

OAV = ((log2 IBDV antibody ELISA titre of bird (%) - log2 breakthrough titre of the vaccine)) x t½) + Age at sampling + Correcting value 0 to 4

In which:
(%) birds is ELISA titre of the bird representing a certain percentage of the flock ready to be vaccinated (75% in this study)
Breakthrough titre is the vaccine take level (500 in this study: the breakthrough of the intermediate plus vaccine)
t ½ is the half life of MDA (3 days)
Age at sampling is age of the birds at sampling.
Correction value 0-4 is extra days when the sampling was done at 0 to 4 days of age. This correction is made because the antibody level starts to decline from the age of 4 days.

Statistic analysis: ELISA titres and their CV in the two types of samples (sera and egg yolk) were compared with the independent samples t-test and paired samples t-test respectively. Statistical significance was set to $p = 0.05$.
The correlation between ELISA titres obtained from chick's sera and those obtained from egg yolk was estimated with a regression analysis. The same statistical tool was used to evaluate the relation between breeder flock age and the difference between the two vaccination timing based on egg yolk and serum titres respectively.
Analyses were carried out with the statistical software MedCalc version 10.2.0.0.

RESULTS

Serum and egg yolk MDA titres: Results are summarized in Fig. 1. Overall, the ELISA titres were high in both types of samples. The difference was not statistically significant between the yolk and serum titres ($p>0.05$ NS), which were well positively correlated ($r = 0.74$, $p<0.05$*) (Fig. 2).
The uniformity of ELISA titres was similar in both types of samples ($p>0.05$ NS). Only the flock n°4 showed a significant discrepancy between the two CVs (51% in egg yolk vs 27% in sera).

Vaccination age estimations: The earliest estimation (13.9 days) was obtained from egg yolk titres in the flock n°1, 50 weeks of age. As for the most deferred estimation (17.3 days), it was obtained from sera in the youngest flock (flock n°7, 34 weeks of age) (Table 1).
The difference in absolute value between the two dates of vaccination was 0.3 days for flock n°3, 4 and 6 to 1.1 days for flock n°1. This gap was not influenced by the age of the parent breeder-flock ($r = 0.04$, $p>0.05$ NS) (Fig. 3).

DISCUSSION

The critical role of the vaccination age estimation in the success of active immunization process against IBD is well established (van den Berg, 2000). This prediction is

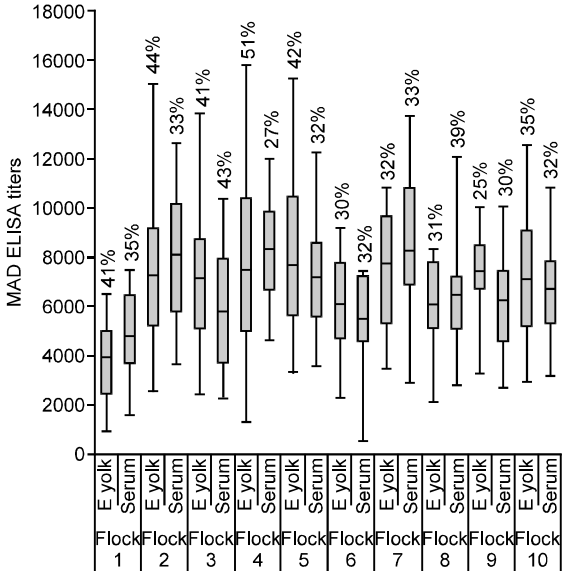

Fig. 1: Eliza titres of MDA in egg yolk and hatched chicks serum for the ten breeder flocks. Data are presented in box and whisker diagram: 1st and 3rd quartile (upper and lower borders of box respectively), mean ELISA titres (short line within the box), highest and lowest ELISA titres (upper and lower whiskers respectively). Percentage values represent the coefficients of variation

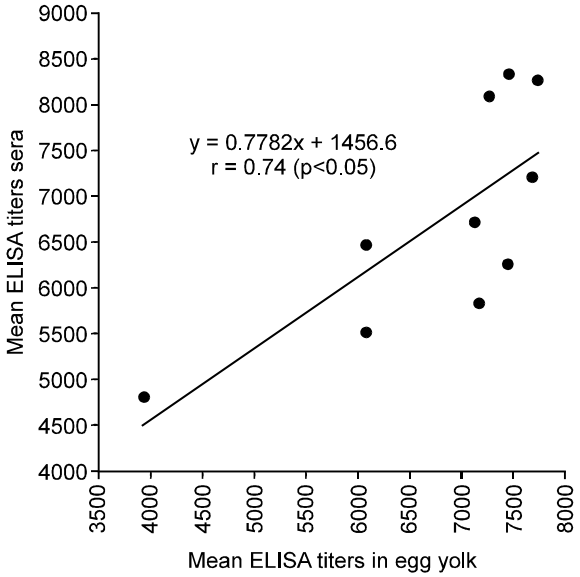

Fig. 2: The positive correlation between ELISA titres in egg yolk and chick's sera

based on serological data obtained by detection of IBDV specific maternal antibodies by ELISA system in newborn chicks (De Wit, 2001), which implies the chicks sacrifice. This method of sampling is impractical, costly

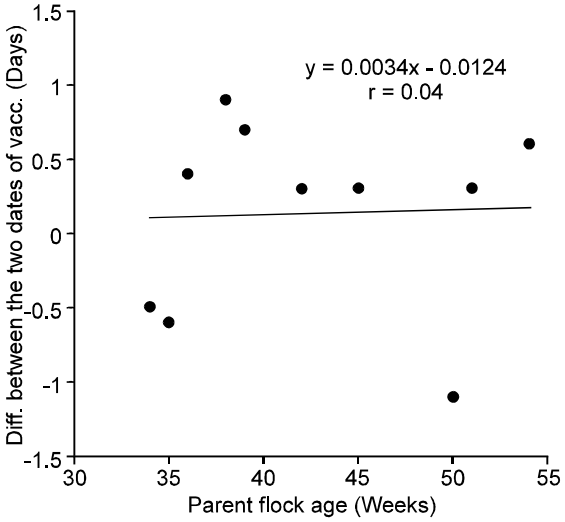

Fig. 3: Absence of correlation between the parent flock age on one hand and the difference between the two dates of vaccination on the other hand, as indicated by the low value of the correlation coefficient

Table 1: Comparison between OAVs based on egg yolk antibodies and those calculated from chick's sera antibodies

		OAV (days)		
Flocks	Parent flock age (weeks)	Egg yolk	Serum	Difference (days)
Flock 1	50	13.9	15.0	-1.1
Flock 2	35	16.8	17.2	-0.6
Flock 3	45	16.2	15.9	+0.3
Flock 4	42	17.1	16.8	+0.3
Flock 5	38	17.1	16.2	+0.9
Flock 6	51	15.8	15.5	+0.3
Flock 7	34	16.8	17.3	-0.5
Flock 8	36	15.9	15.5	+0.4
Flock 9	54	16.3	15.7	+0.6
Flock 10	39	16.5	15.8	+0.7

and invasive. It represents thereby, a limit to the systematic calculation of the OAV before the introduction of the chicks.

To study the possibility of using egg yolk antibodies instead of chick's plasma antibodies for predicting IBD vaccination timing, we have compared the antibody titres in these two types of samples as well as the vaccination age estimations they lead to.

Globally, the antibody titres in both types of samples were relatively high. For some breeder-flocks, this could be explained by outbreaks of IBD occurring during young age (Lukert and Saif, 2009; Block *et al*., 2007), which are frequently observed in our region (North-East of Algeria). Such contaminations would be an excellent priming before boosting hens with an oil-emulsion vaccine near the point of lay.

Our results show that, for the same flock, ELISA titres in egg yolk and those in the sera of the chicks, were similar (p>0.05 NS). This corroborates the results of De Wit *et al.* (2001) which show a lack of a significant difference between egg yolk titres and newborn chick's serum titres and that, for five different commercial ELISA kits, including IDEXX kit (the one used in this study). Furthermore, antibody titres in both types of samples were well correlated (r = 0.74, p<0.05). An acceptable correlation was also demonstrated by Jungback and Finkler (1996) between the level of residual antibodies in embryonated eggs and those in hatched chicks.

Regarding the uniformity of ELISA titres, the CVs of antibody levels in both types of samples were not significantly different (p>0.05). With the exception of the flock n°4, for all the other flocks and whatever the type of samples, a single vaccination would be sufficient (even if the CVs of egg yolk titres in some flocks were above 40%, these titres remain sufficiently homogeneous to require only a single vaccination (De Wit, 2001).

The absolute value of the difference between OAV determined from egg yolk titres and those based on serum titres, never exceed 1 day (only the flock n°1 has recorded 1.1 day as a difference). Such a gap would be without impact on the risk of a vaccine failure. Indeed, Block *et al.* (2007) have highlighted seroconversion in all broiler flocks IBD-vaccinated after, at, or one day before the estimated optimal time point (evaluated by Deventer formula) and only the flocks vaccinated more than 1 day before OAV had a delayed or non-detectable immune response until slaughter.

Finally, the difference between vaccination age estimations does not seem to depend on the parent flock age (r = 0.04, p>0.05 NS).

Three arguments are in favor of the possibility of substituting chick's serum by egg yolk in predicting the OAV against IBD:

- ELISA titres in both types of samples were not only similar (p>0.05 NS), but also well correlated (r = 0.74, p<0.05).
- CVs in both types of samples, as indicators of titre uniformity, were generally very close (p>0.05 NS).
- The difference between the two vaccination age estimations was minimal (from 0.3 to 1.1 days).

Compared to blood samples, fertilized eggs are daily available and their collect is easier, not stressful and less costly. The only inconvenient of using yolk antibodies is the method of preparing yolk for antibody testing. This method can be efficiently replaced by a simple dilution method with no mixing or extraction as it was performed in this experience and demonstrated by many other studies (Mohammed *et al.*, 1986; Haddad *et al.*, 1997).

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