

DETERMINATION OF THE BREAKTHROUGH ELISA TITRE OF LIVE IBD IMMUNE COMPLEX VACCINE STRAINS

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INTRODUCTION

The estimation of the optimal vaccination time is of major importance when conventional infectious bursal disease (IBD) live attenuated vaccines are being used. Employing the “Deventer formula” may help to estimate this time for a specific flock based on the maternally derived antibody level, the genetic background of the chicken, and the IBD vaccine strain. To apply this formula, the breakthrough ELISA titre of the vaccine strain must be known. This titre is specific for each vaccine strain, in accordance with its level of attenuation. In the case of immune complex vaccines, there is no need for calculation of the vaccination time since the vaccine is capable of self-adaptation to the individual level of maternally derived antibodies (MDA) of each chick in the vaccinated flock. Nevertheless, the breakthrough ELISA titre of the vaccine strain present in the formulation can be of interest in order to estimate the time when the vaccine virus is able to start to replicate in the bursa of Fabricius.

The objective of this study was to determine the breakthrough titre of strain 1052, the vaccinal strain of GUMBOHATCH®. Furthermore, this breakthrough titre was compared with that obtained in the same study for another immune complex vaccine strain (W2512). The determination of the breakthrough ELISA titre was based on the detection of virus replication in birds with known levels of MDA at the time of inoculation, by means of reverse transcription PCR (RT-PCR).

MATERIALS AND METHODS

A total of 163 healthy day-old broiler chicks were distributed into 3 groups based on ELISA titre (CIVTEST® AVI IBD) at 7 days of age. 2 groups were vaccinated by drinking water with an equivalent dose of each of the immune complex vaccine strains, while a third group was kept as a non-vaccinated control group (Table 1). Birds were inoculated at 15 days of age, as a result of Deventer formula calculation.

Group	Treatment	Number of animals
1	Strain A (1052)	64
2	Strain B	71
3	Control (PBS)	28

Table 1. Type of vaccines and number of samples studied

All the chicks were humanely killed at 5 days post-inoculation, and bursa of Fabricius imprints on FTA cards were collected to perform RT-PCR. Total RNA was extracted and purified using the RNeasy Mini kit (QIAGEN). RNA extracted was transcribed into cDNA using the OneStep RT-PCR Kit (QIAGEN). Subsequently, conventional PCR was used to amplify a 248-bp sequence of the hypervariable region of the VP2 gene of IBDV¹.

RESULTS

No differences between groups were detected in the ELISA titre at vaccination day (figure 1, Kruskal-Wallis test, *P* value=0.161).

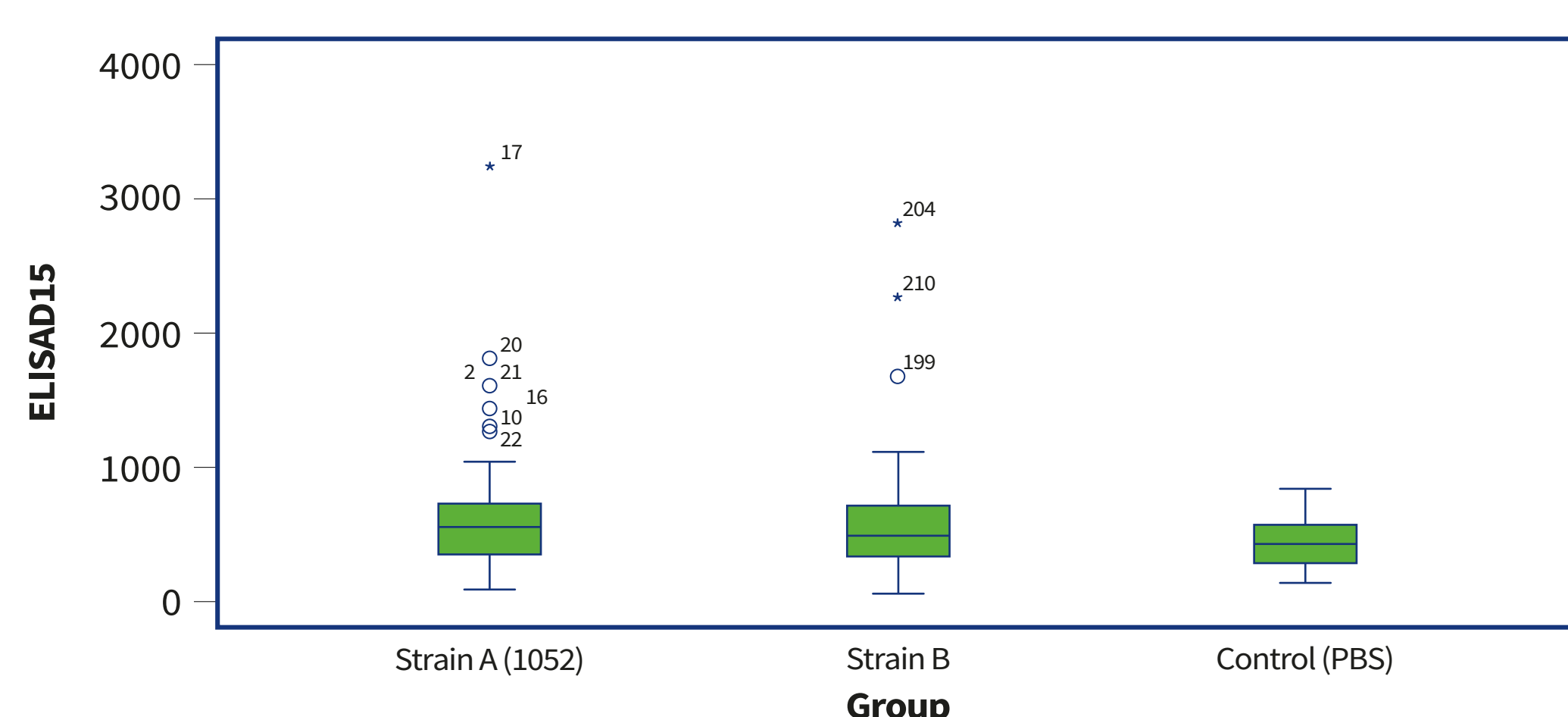


Fig. 1. ELISA titre* distribution at vaccination day

Data are presented as a box plot: The median marks the mid-point of the data and is shown by the line that divides the box into two parts; the lower and upper limits of the box represent the first and third quartiles, respectively; the upper and lower whiskers represent the minimum and maximum values of the distribution, except for outliers (represented as “” or “o”).

Forty-four birds (68.8%) from the group vaccinated with GUMBOHATCH® and 25 chicks (35.2%) from the group vaccinated with the alternative immune complex vaccine strain were positive for IBDV, while all the birds from the non-vaccinated group remained negative (table 2).

Group	PCR	Frequency	%
Strain A (1052)	-	20	31.2
	+	44	68.8
	Total	64	100.0
Strain B	-	46	64.8
	+	25	35.2
	Total	71	100.0
Control (PBS)	PCR -	28	100.0

Table 2. RT-PCR results 5 days post-vaccination

Mean ELISA titres were calculated for positive birds, in a similar way to that done by Kouwenhoven in the determination of breakthrough titres for “hot” vaccines (Table 3).

Group	Mean ELISA titre
Strain A (1052)	513.79
Strain B	405.56

Table 3. Mean ELISA titre at vaccination day (breakthrough ELISA titre) of birds positive for IBDV by RT-PCR 5 days post-vaccination

DISCUSSION

The breakthrough titre of strain 1052 was somewhat higher than that of a reference strain included in the formulation of the alternative immune complex vaccine (514 vs 406). These results are in accordance with previous experimental and field observations, where an earlier vaccine virus replication was observed with GUMBOHATCH® compared to other commercial immune-complex vaccines.

REFERENCES

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