Research note

Monitoring of vaccine take by quantitative real-time polymerase chain reaction following different Marek's disease vaccination programs in future broiler breeders

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Abstract

The objective of this study was to compare different Marek's disease (MD) vaccination programs in conventional broiler breeder pullets utilizing quantitative real-time polymerase chain reaction (qPCR) on feather pulp and spleen samples. Sample collection utilized a novel transport system of antigenic material from the desired samples. Vaccine programs compared *in ovo*, subcutaneous at hatch or intramuscular at hatch injection of a recombinant Marek's and IBD vaccine associated to a MD Rispens vaccine, and injected twice as part of broiler breeder vaccination program. Double vaccinations using VAXXITEK® HVT+IBD and Rispens strain mixed vaccines did not impair the vaccine virus replication kinetics *in vivo*.

Introduction

Determination of vaccine take is a quality control step in vaccination principals, but it has been difficult in the past for MD vaccines. Quantification of serotype 1 MD vaccines *via* real-time PCR can be used to assess the efficacy of the vaccination procedure by evaluating the MD vaccine load in a representative number of chickens [1]. Flinders Technology Associates (Whatman plc, Maidstone, UK) paper cards are impregnated with a patented chemical formula that lyses the cells and denatures proteins upon contact. The nucleic acids are protected against nucleases, oxidation, UV damage, and microbes. The novel method of using FTA cards for the collection, shipment, and documentation of biologic samples has been demonstrated as a helpful tool in detection of several poultry pathogens including MD [1], [2]. The present study was designed to evaluate the efficacy of three different programs using qPCR to quantify the amount of MD vaccine in vaccinated populations. All three programs utilized the HVT-IBD vector vaccine with Rispens and only varied in their route of administration and the number of times administered.

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Material & methods

160 commercial broiler breeder pullets (Cobb-Vantress, Putten, Netherlands) were placed into one of four groups and vaccinated according to the study design (Table 1).

Mareks disease PCR

Feathers were collected from each bird at 3, 5 then 8 weeks of age (Figure 1). Each fresh feather tip was rubbed onto the surface of a paper card. Spleens from 7 chickens for each group were sampled at 7 days of age, then at 3, 5 and 8 weeks of age. Each sampled spleen was rubbed onto the surface of FTA cards. All feather tip and spleen samples were shipped to North Carolina State University and then processed for Marek's disease qPCR using published procedures [1]. 20 commercial chickens were used as controls. Blood and tissue sampling procedure and dates were identical to the test groups.

Serology

Blood sampling was performed at 3, 5 then 8 weeks of age. Sera were obtained after centrifugation of the collected blood tubes. Sera samples were analyzed using the commercial ELISA kit IBD PROFlock® plus (Synbiotics Corp., Kansas City, MO, USA).

Statistical Analysis

Kruskall-Wallis non-parametric test.

Results / Discussion

For qPCR HVT (Tables 2, 3, 4 & 5; Figures 2, 3, 4 & 5), some erratic points were observed in spleen and feather pulp analysis. Despite a significant difference at 8 weeks of age in feathers being found, the authors feel this is not relevant. Globally, it appears clear that there were no interferences in HVT replication. qPCR CVI988 (Tables 6, 7, 8 & 9; Figures 6, 7, 8 & 9), like for the HVT analysis, the CVI988 qPCR showed some erratic points at different ages too. The CVI988 erratic points were from the same animals as HVT qPCR. No significant difference was found and we can consider that there were no interferences in HVT replication.

There was a significant difference at 3 weeks of age in ELISA IBD (Figure 5) explained by the normal decline of maternal antibodies. An incremental increase of normal antibody was detected by 8 weeks of age, following normal seroconversion patterns. The antibody levels were considered for ELISA IBD plus (Figure 6) as protective, showing seroconversion in all groups, with two significant differences at 3 and 5 weeks of age. At 3 weeks of age, a slightly lower seroconversion was found in the VAXXITEK HVT+IBD+ Rispens injected in ovo. Later seroconversion could be due to the route administration of the vaccine. Despite the significant difference, the antibody levels are still protective

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and classifies as linked to post-vaccination seroconversion. At 5 weeks of age, a strongly lower ($p<0.001$) antibody level was detected in group vaccinated twice at D1 with a double dose of VAXXITEK HVT+IBD + Rispens. Antibody levels were not reduced to IBD in that group, so protection was not sacrificed against IBD.

Conclusion

There were no vaccine interferences in Marek's protection. Interference could have been playing a role in this scenario due to the dual vaccinations at the same age. Further study may be necessary, especially with dual vaccination programs using one VAXXITEK HVT+IBD application with a dual Rispens.

References

[1] Cortes A.L., Montiel E.R., Lemiere S. & Gimeno I., 2009, Validation of Marek's disease diagnosis and monitoring of Marek's disease vaccines from samples collected in FTA cards, Avian Dis., 53, 510-516.
[2] Moscoso H., Alvarado I., & Hofacre C., 2006, Molecular analysis of infectious bursal disease virus from bursal tissues collected on FTA filter paper, Avian Dis., 50, 391–396.

Table 1: Study design.

Group	Number	Vaccination *in ovo*	Vaccination at day-old (sub-cutaneous route)	Vaccination at day-old (intramuscular route)
G1	40	VAXXITEK HVT + IBD + Rispens vaccine	-	-
G2	40	-	VAXXITEK HVT + IBD + Rispens vaccine	-
G3	40	VAXXITEK HVT + IBD + Rispens vaccine	VAXXITEK HVT + IBD + Rispens vaccine	-
G4	40	-	VAXXITEK HVT + IBD + Rispens vaccine	VAXXITEK HVT + IBD + Rispens vaccine

Table 2: HVT qPCR on spleens - quantification.

Group	Week 1	Week 3	Week 5	Week 8
G1	1.00*E+0	2.00*E+0	4.00*E+0	4.00*E+0
G2	7.00*E-1	1.00*E+0	9.00*E+0	5.00*E+0
G3	7.00*E-1	3.00*E+0	5.00*+0	8.00*E+0
G4	2.00*E+0	1.00*E+1	7.00*E+0	8.00*E+0
p	0.402	0.183	0.638	0.881

Table 3: HVT qPCR on spleens – proportions of positives.

Group	Week 1	Week 3	Week 5	Week 8
G1	4/5	3/5	5/5	5/5
G2	5/5	4/5	5/5	5/5
G3	4/5	5/5	5/5	5/5
G4	5/5	5/5	5/5	5/5

Table 4: HVT qPCR on feathers – quantification.

Group	Week 3	Week 5	Week 8
G1	3.94*E-1	1.18*E+2	9.50*E-1
G2	5.14*E+0	1.96*E+1	3.27*E-1
G3	3.73*E+1	5.27*E+0	1,75*E+0
G4	2.55*E+1	3.11*E+1	2.35*E-1
p	0.052	0.907	0.030*

Table 5: HVT qPCR on feathers – proportion of positives.

Group	Week 3	Week 5	Week 8
G1	2/5	4/5	4/5
G2	5/5	5/5	3/5
G3	5/5	4/5	5/5
G4	4/5	5/5	3/5

Table 6: CVI988 qPCR on spleens - quantification.

Group	Week 1	Week 3	Week 5	Week 8
G1	1.99*E+2	6.09*E+2	1.01*E+3	2.94*E+2
G2	1.42*E+2	9.16*E+1	1.08*E+3	1.56*E+3
G3	1.58*E+2	1.66*E+3	2.13*E+2	1.45*E+3
G4	2.46*E+2	1.32*E+4	7.89*E+2	8.42*E+2
p	0.894	0.593	0.359	0.581

Table 7: CVI988 qPCR on spleens – proportions of positives.

Group	Week 1	Week 3	Week 5	Week 8
G1	3/5	2/5	2/5	1/5
G2	2/5	2/5	3/5	3/5
G3	2/5	3/5	2/5	1/5
G4	3/5	1/5	5/5	2/5

Table 8: CVI988 qPCR on feathers - quantification.

Group	Week 3	Week 5	Week 8
G1	1.00*E+4	1.66*E+5	2.79*E+3
G2	2.30*E+4	1.99*E+5	3.28*E+2
G3	1.21*E+5	6.78*E+3	6.16*E+2
G4	5.94*E+5	1.26*E+6	1.79*E+3
p	0.804	0.142	0.650

Table 9: CVI988 qPCR on feathers – proportions of positives.

Group	Week 3	Week 5	Week 8
G1	2/5	2/5	3/5
G2	3/5	4/5	1/5
G3	3/5	1/5	2/5
G4	2/5	5/5	2/5

Table 10: Classic IBD ELISA monitoring.

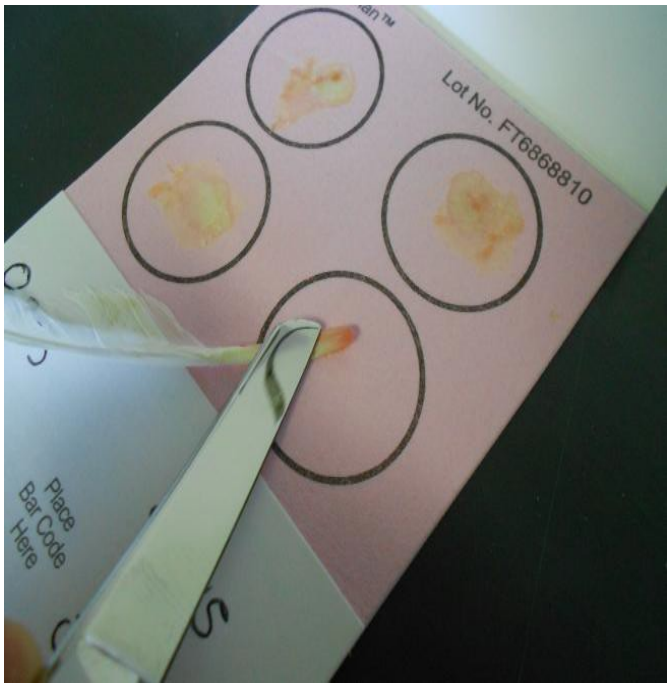
	Week 1	Week 3	Week 5	Week 8
G1	1193	440	81	560
G2	959	615	136	607
G3	1457	695	91	546
G4	1509	166	217	466
p	0.894	0.020*	0.307	0.415

Table 11: IBD Plus ELISA monitoring.

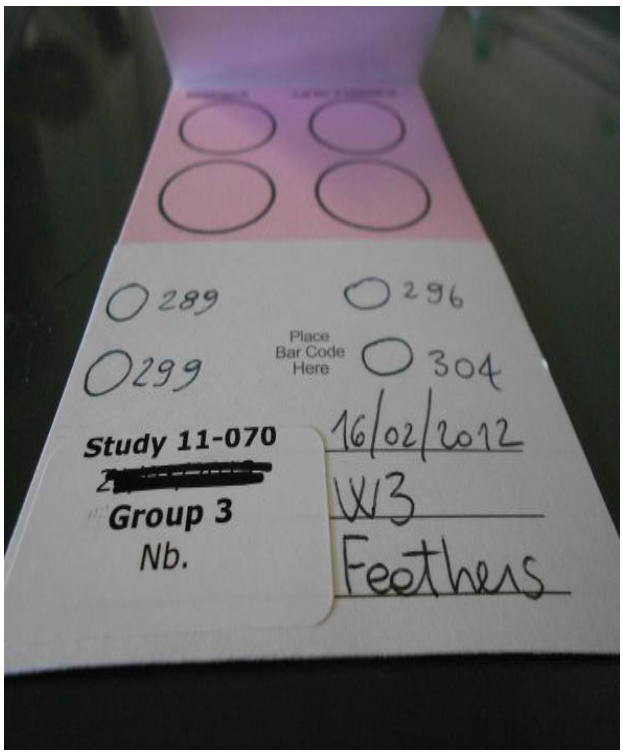
	Week 1	Week 3	Week 5	Week 8
G1	9536	6689	11861	10883
G2	8988	9194	12741	11692
G3	9748	8261	11337	11495
G4	9442	7963	7719	11188
p	0.809	0.010	0.000	0.142

Figure 1: Tissue sample collection using FTA cards.

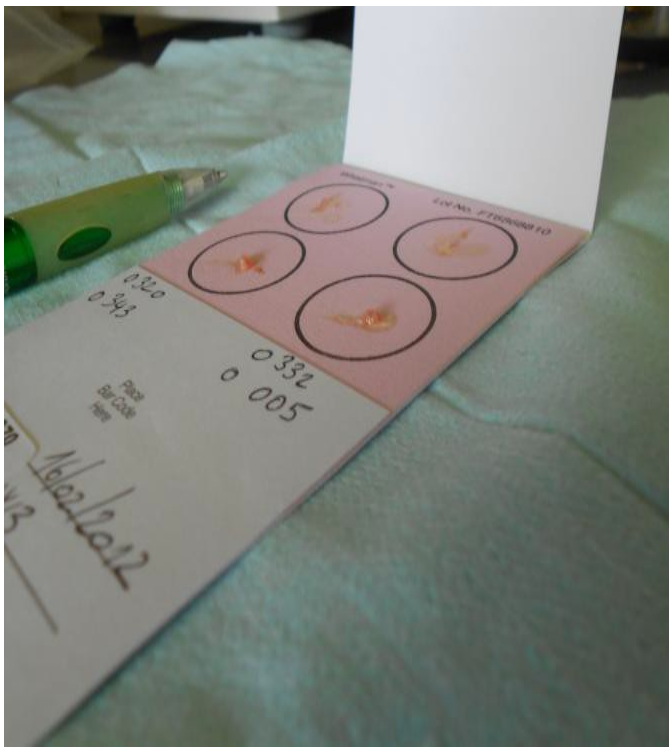
Feather sampling and identification



Extraction of the feather pulp



FTA card identification



FTA card identification

Figure 2: HVT qPCR on spleens - quantification.

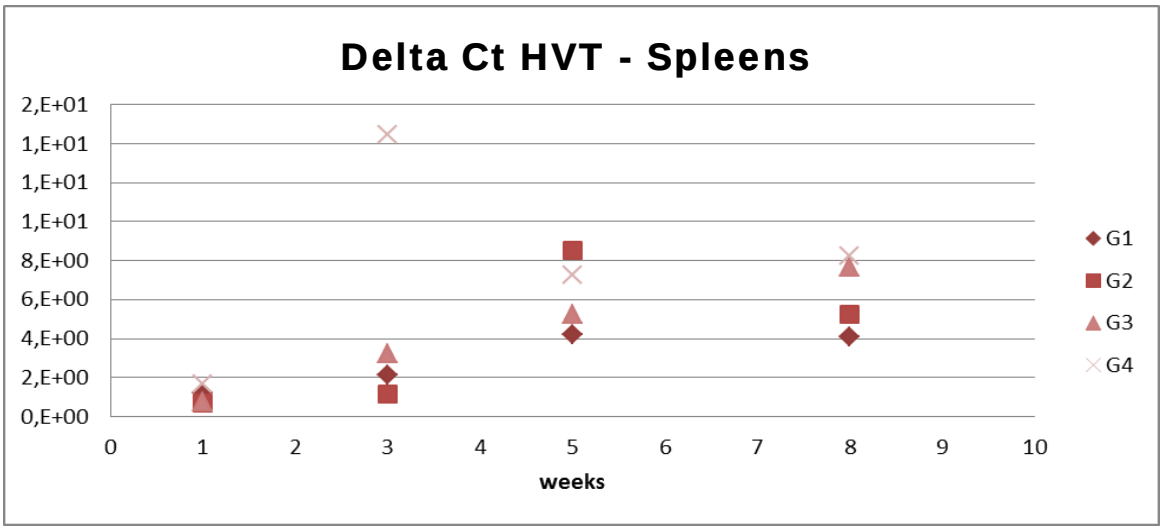


Figure 3: HVT qPCR on spleens- proportion of positives.

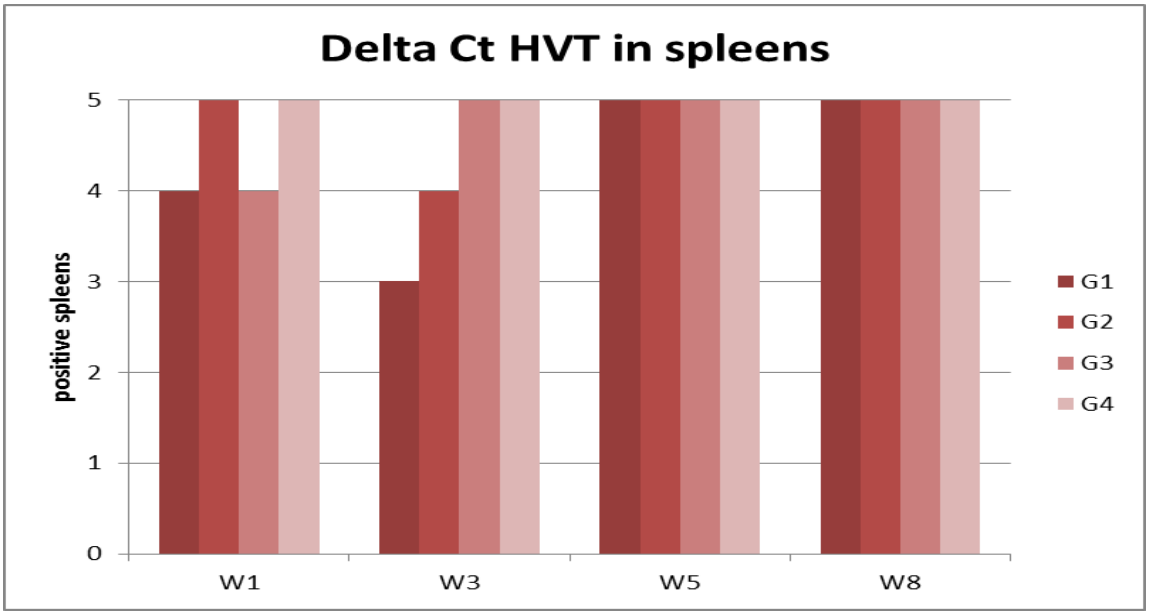


Figure 4: HVT qPCR HVT on feathers - quantification.

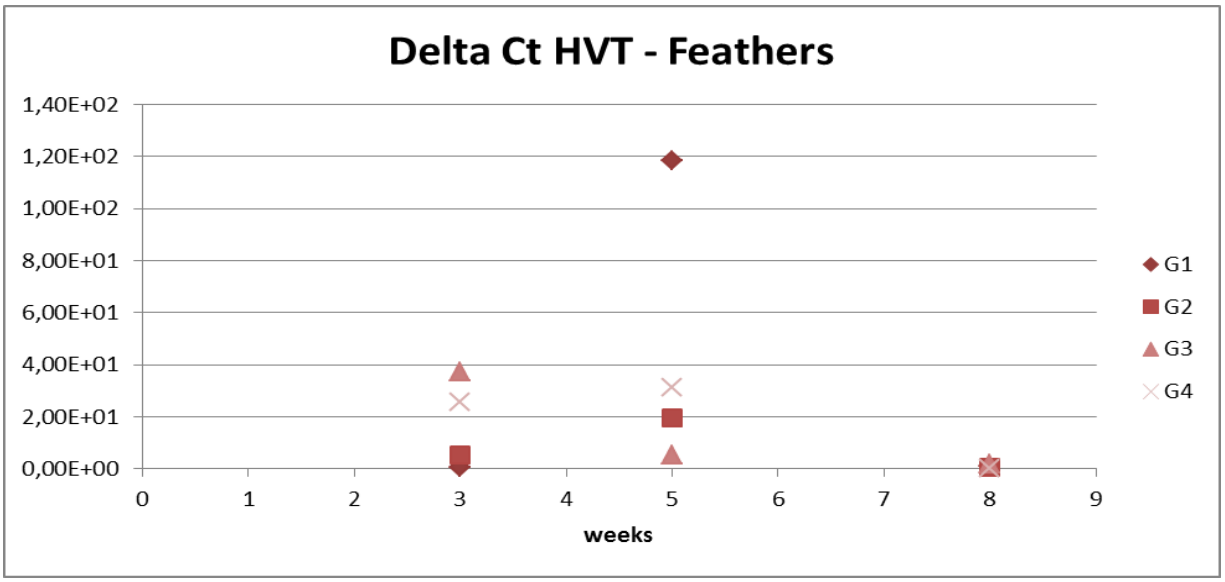


Figure 5: HVT qPCR HVT on feathers – proportion of positives.

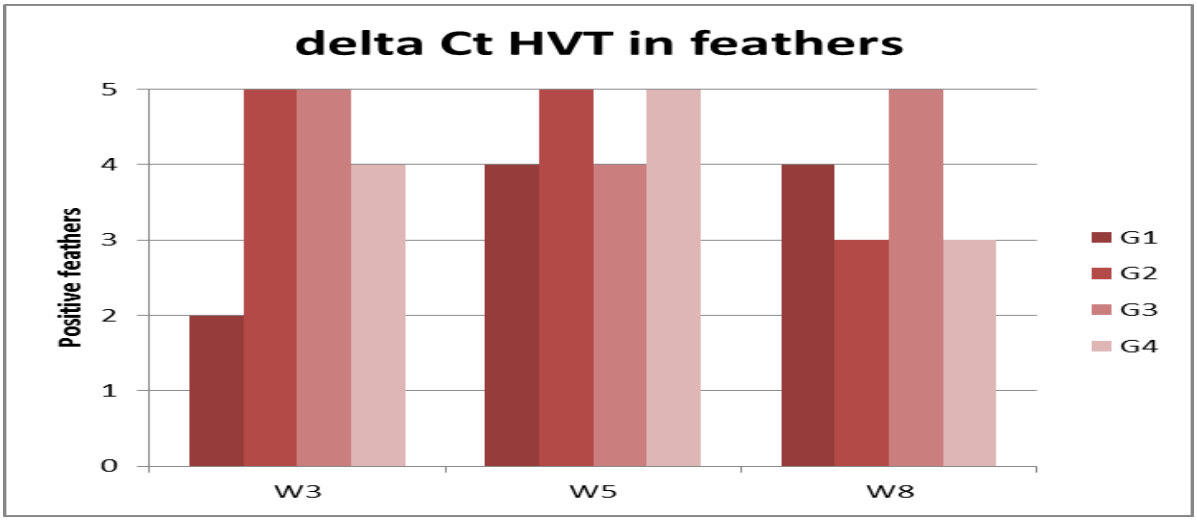


Figure 6: CVI988 qPCR on spleens - quantification.

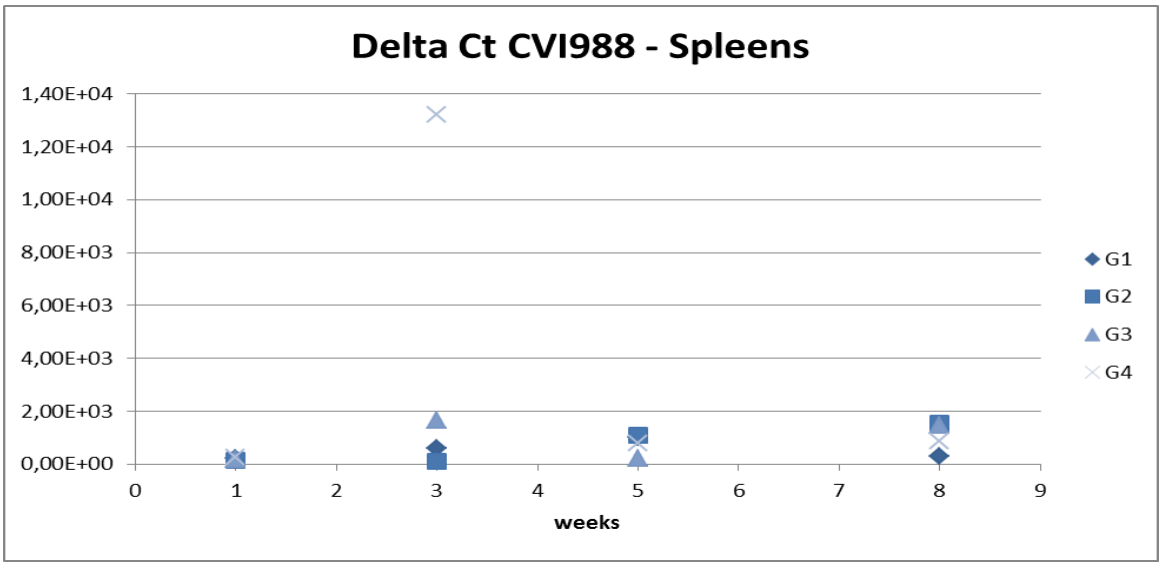


Figure 7: CVI988 qPCR on spleens – proportion of positives.

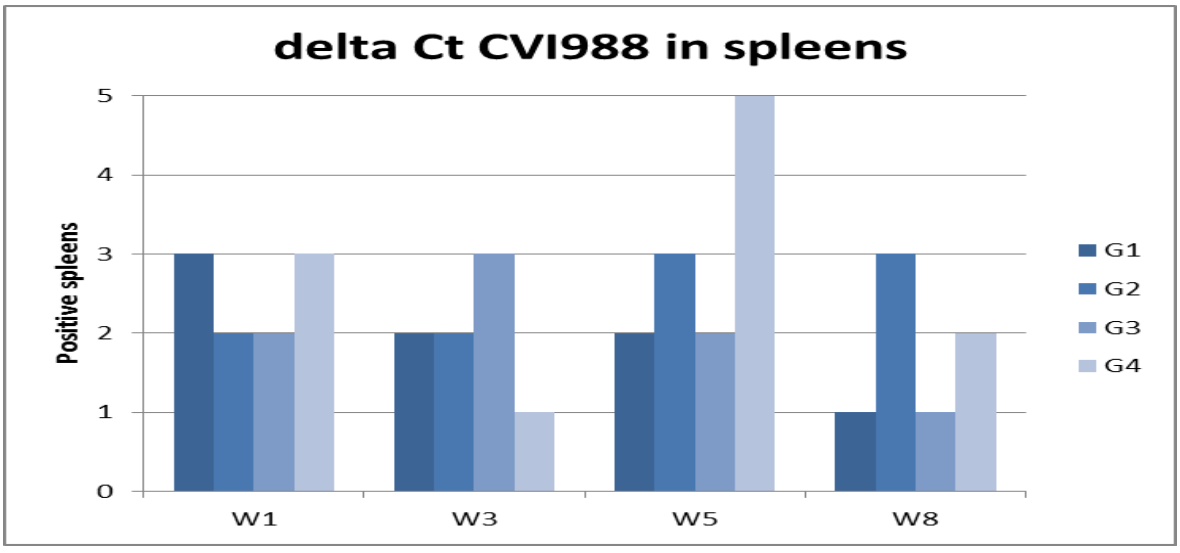


Figure 8: CVI988 qPCR on feathers – quantification.

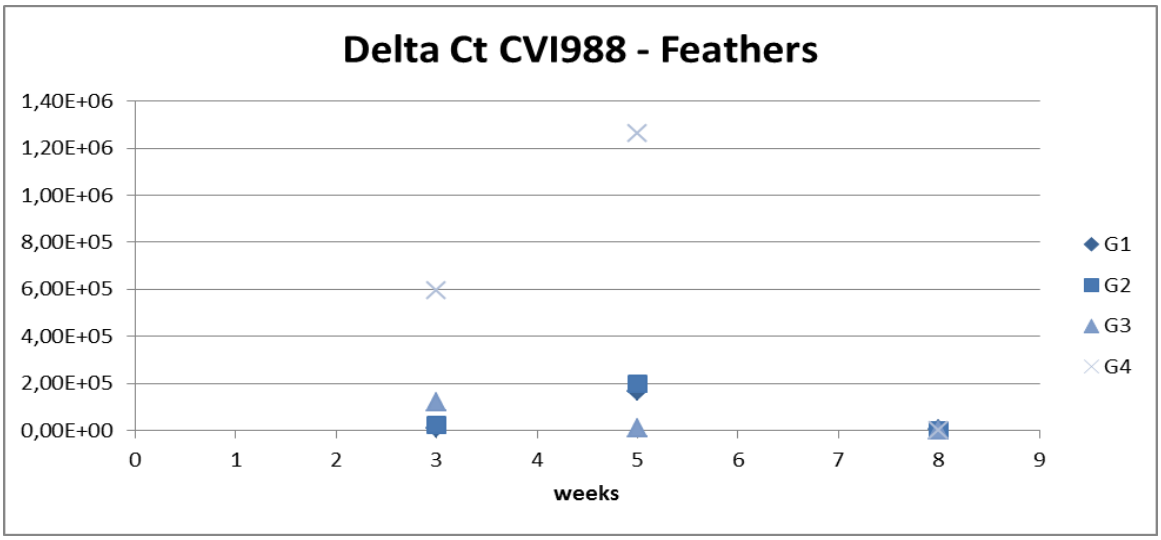


Figure 9: CVI988 qPCR on feathers – proportion of positives.

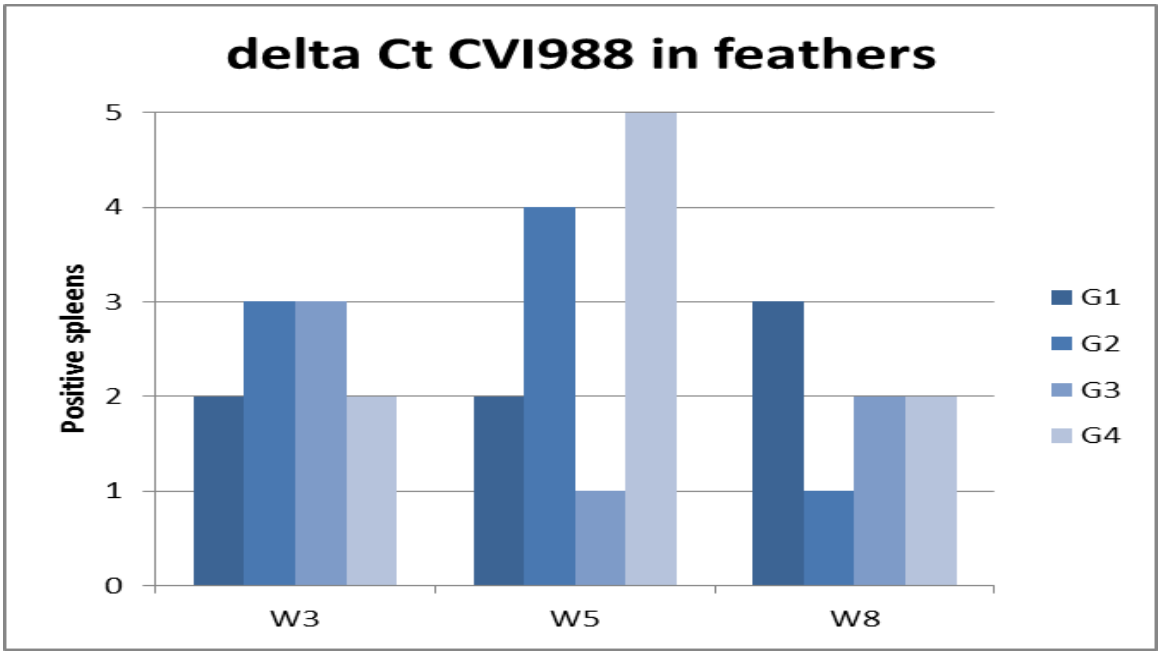


Figure 10: Classic IBD ELISA monitoring.

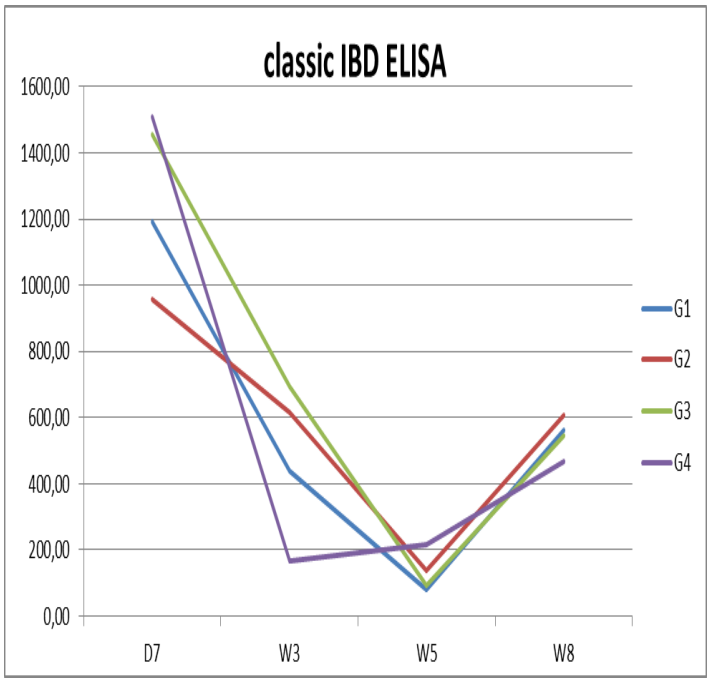


Figure 11: IBD plus ELISA monitoring.