Optimising infectious bronchitis vaccination of laying hens for maximum egg shell quality

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by Juliet R. Roberts

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Foreword

This study was designed to evaluate different vaccination protocols for infectious bronchitis virus in laying hens. Age at first vaccination, route of vaccine administration, regular revaccination during lay, and timing of revaccination in relation to an induced moult was investigated. Protection of birds afforded by different vaccination protocols was evaluated by challenging birds with the standard Australian challenge virus, T-strain infectious bronchitis virus. The degree of protection of birds was assessed by monitoring production, egg internal quality, egg shell quality, excreta moisture, blood electrolytes, and kidney histology.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Acknowledgments

The Preliminary Trial and Trial 1 were conducted as the Ph.D. project of Mr. Abrani Sulaiman (submission date March 2004). The kidney histology in the Preliminary Trial was conducted by Ms. Megan Jolly for her Honours Project (2001). Trial 2 formed part of the Ph.D. project of Ms. Megan Jolly (submission date January 2005). Copies of the two Ph.D. theses will be provided to the AECL.

Mrs Wendy Ball provided excellent technical assistance at all stages of the project. Her many years of experience with infectious bronchitis virus made her input to this study invaluable.

Dr. Roger Chubb assisted with the setting up of the agar-gel-precipitation test and contributed to informative discussion. The late Associate Professor Rob Cumming provided the inspiration for this study.

Mr. Gary Taylor, Ms. Janelle McFarlane and Mr. David Creed provided technical assistance.

IBV antibody titres were measured by Birling Avian Laboratories and Mr. Peter Cowling of the International Avian Health Group at the University of Melbourne, on a fee-for-service basis.

The Preliminary Study presented here was funded by the University of New England through its support for Postgraduate Students.

The financial support of the Australian Egg Corporation Limited (formerly the Egg Program of the RIRDC) for the project and for providing a Postgraduate Scholarship to Ms. Megan Jolly is gratefully acknowledged.

Mr. Sulaiman was supported by a Postgraduate Scholarship from AusAid.

About the Author

Juliet Roberts is an Associate Professor in Animal Physiology at the University of New England. Her research interests include factors affecting egg internal quality and egg shell quality, including disease, avian physiology and avian nutrition.

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Abbreviations

AAA	A3 vaccine at day old, 4 weeks and 14 weeks of age
AVA	A3 vaccine at day-old, VicS vaccine at 4 weeks and A3 vaccine at 14 weeks of age
A3	A3 vaccine administered by eyedrop
A3 spray	A3 vaccine administered by coarse spray
A3 water	A3 vaccine administered in water
AE	avian encephalomyelitis
Ca	calcium
Ca^{++}	ionised calcium
Cl	chloride
CD	ciliostatic dose
EID	embryo infective dose
ELISA	enzyme-linked immunosorbent assay
ERBF	effective renal blood flow
ERPF	effective renal plasma flow
F.E.	fractional excretion
GFR	glomerular filtration rate
Hct	haematocrit (also known as packed cell volume)
IB	infectious bronchitis
IBV	infectious bronchitis virus
K	potassium
LSD	least significant difference
Na	sodium
N1/88	a strain of IBV
NS	not statistically significantly different
P	probability
PAH	para-aminohippuric acid
S.E. (or SEM)	standard error of the mean
T	a strain of IBV
T.L.	tubular load
VVV	VicS vaccine at day old, 4 weeks and 14 weeks of age
VAV	VicS vaccine at day-old, A3 vaccine at 4 weeks and VicS vaccine at 14 weeks of age
VicS eye	VicS vaccine administered by eyedrop
VicS spray	VicS vaccine administered by coarse spray
VicS water	VicS vaccine administered in water

Executive Summary

Preliminary Trial

The Preliminary Trial reported here was funded by the University of New England, prior to the commencement of the present project, as part of a postgraduate project. The effect of age at first vaccination and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) was investigated in cockerels. ISA Brown cockerels were reared in isolation pens at the University of New England, Armidale, NSW. There were seven experimental groups, Control (no vaccination), Eyedrop I (vaccination by eyedrop at day-old), Spray I (vaccination by coarse spray at day-old), Water I (vaccination by coarse spray at day-old), Eyedrop II (vaccination by eyedrop at two weeks), Spray II (vaccination by coarse spray at two weeks), Water II (vaccination in water at two weeks). For all vaccinated groups, the vaccine used was Webster's VicS strain IBV. At 8 weeks of age, birds were re-vaccinated, by the same routes as at day old or two weeks (except Control Group). At 11 weeks of age, birds were exposed to T-strain IBV. The results indicate that both ages of vaccination (day-old and two weeks of age), and all routes of vaccination (eyedrop, coarse spray and water vaccination) were effective in protecting most birds against the effects of exposure to T strain IBV. The unvaccinated birds reacted to the T strain IBV with a large increase in antibody titres, respiratory symptoms, wet droppings and mortality. In addition, there was significant enlargement of both kidneys and histopathological damage to the kidneys, an indication of the nephropathogenic effects of T strain IBV in birds which are not protected by an adequate vaccination program.

Trial 1

Different vaccination protocols for infectious bronchitis (IB) virus were administered to Isa Brown laying hens. During the rearing phase, there were few differences among the vaccination groups and there was no evidence that either vaccine strain used (VicS, A3) caused a "set-back" effect on the birds. The control group remained negative for IBV antibodies until deliberately vaccinated at 14 weeks of age.

Half the birds were revaccinated regularly during lay whereas the other birds were not vaccinated beyond 14 weeks of age. Initial vaccination treatment had some effects on production and egg quality. The control group had lower egg weight and lighter shell colour and shell weight. The birds that received the A3 vaccine in water initially had better shell quality but lower albumen quality. Regular revaccination during lay had negative effects on both production and egg quality. However, the birds that were revaccinated regularly had slightly higher egg weight.

The IB antibody titres were greatest at 6 and 16 weeks for both revaccinated and non-revaccinated birds. However, regular revaccination of birds beyond 14 weeks of age had no significant effect on IB antibody titre levels. These results suggest that there may be little advantage in regular revaccination of birds for IB, provided that birds have been properly vaccinated during rearing.

At 57 wks of age, birds were placed into an induced moult and were revaccinated for IBV either before or after the moult. Revaccination occurred at 62 weeks of age. Production was lower during the moult study in the birds that were revaccinated regularly during lay and the control and VicS eyedrop groups. Egg shell quality was better in the birds that were revaccinated prior to moult. IB titres increased following revaccination.

When birds were challenged with T-strain IBV at 77 weeks of age, the main effect was an increase in the incidence of abnormal eggs and a large rise in IBV antibody titres in most treatment groups. There were few effects on kidney histology.

Trial 2

In Trial 2, four different strains of birds were studied, Isa Brown, HiSex Brown, HyLine Brown and HyLine Grey. All birds received three vaccinations for IBV during rearing and for most birds these vaccinations were at day-old, 4 weeks and 14 weeks. Two vaccine strains were used, VicS and A3, in various combinations of either one or both strains.

For the challenge study at 25 weeks of age, birds received their third vaccination at either 14 or 18 weeks, to investigate any possible deleterious effects of vaccination close to point of lay. Birds were challenged with T-strain IBV by eyedrop of 1 in 5 birds. Production was not affected but, for the whole flock, there was an immediate decline in egg internal quality after challenge with the virus. Shell quality also declined as evidenced by both objective measurement and visual assessment. There were no major effects due to the strain of vaccine that the birds received at day old. The revaccination of birds at 5% lay (18 weeks old) resulted in an increased decline in egg quality measurements when birds were challenged with T-strain. It appears that the third vaccination is best given several weeks prior to the onset of lay.

For the challenge at peak of lay, when birds were 41 weeks of age, two challenge viruses were used, T-strain and N1/88. There was a decline in production with T-strain IBV. There was also a decline in egg shell quality, especially for the T group. An unexpected finding was that albumen quality was not adversely affected by either challenge strain. Manure moisture increased in all experimental groups. Plasma electrolyte concentrations were lower and haematocrit higher following challenge and IBV antibody levels increased significantly. Only minor histopathological damage was found in the kidneys and only in the birds that were exposed to T-strain IBV.

When birds were challenged with T-strain IBV at 62 weeks of age, there were some deleterious effects. Production dropped and egg shell quality declined. Again, there were no negative effects on albumen quality, an unexpected finding. Haematocrit increased but there were very few effects on plasma electrolyte levels. Manure moisture increased and IBV antibody titres increased. There were some histopathological changes in the kidneys of the challenged birds.

When kidney function was studied in detail in birds which had not been vaccinated since 14 weeks of age and were challenged at 70 weeks of age, there were relatively few effects. Mild histopathological changes were seen in the kidney of just one of 5 challenged birds that were investigated.

Comparison of Serological Methods

Measurements of infectious bronchitis virus (IBV) antibody titres by IDEXX IBV antibody ELISA were compared with the results of agar-gel precipitation (AGP) and serum neutralization (SN) tests. ELISA was more sensitive at detecting antibodies following vaccination during rearing. The percentage of samples testing positive by AGP and SN increased as the ELISA IBV antibody titre increased. Although it is not clear at what antibody titre level birds are protected against intercurrent infection, a mean IBV antibody titre measured by IDEXX ELISA of 439 correlated with a high level of protection against exposure to T-strain IBV. A comparison of two different IBV antibody ELISA kits showed a significant linear correlation although individual samples did not always correlate closely. While ELISA will continue to be the most practical method commercially, it is important that producers be able to interpret antibody titres from ELISA in terms of the level of protection of the flock.

Overall Conclusions

The results of Trial 1 suggested that there were some disadvantages and few advantages of regular revaccination during lay, provided that birds had been properly vaccinated during rearing. However, the results from Trial 2 of challenge with T-strain IBV at 25, 41 and 62 weeks of age, in birds that had not been revaccinated regularly during lay, generally support the benefits of regular revaccination during lay.

More information is required about the correlation between blood IB titre levels measured by different means, and protection of birds against intercurrent IB infection, before definitive recommendations can be made to the Australian industry.

General Introduction

The infectious bronchitis virus (IBV) and its deleterious effects were first recognised in the United States in the early 1930s (Schalk & Hawn, 1931 in Cavanagh & Naqi, 1997). The presence of IBV in Australian flocks was first identified by the late Associate Professor Robin Cumming, in 1962, as the infectious agent associated with respiratory disease and uraemia. Drs Rob Cumming and Roger Chubb conducted a series of experiments and field trials in the 1960s and 1970s to describe the effects of IB in Australian flocks. These researchers also developed methods for isolating and detecting the virus. In the late 1970s and early 1980s, Wadey and Faragher (1981a,b) used cell culture techniques to identify different subtypes of IBV.

This work was furthered in the late 1980s and the 1990s by Dr. Jagoda Ignjatovic and colleagues (Ignjatovic & McWaters, 1991; Ignjatovic & Ashton, 1996; Ignjatovic *et al.*, 1997; Ignjatovic & Sapats, 2000; Ignjatovic *et al,* 2001) who developed molecular techniques for characterisation of the different strains of the virus and described their pathogenicity. Ignjatovic and colleagues identified a shift in the prevalent IBV strains from highly nephropathogenic in the 1960s and 1970s to predominantly respiratory in the 1980s and 1990s (Ignjatovic *et al*., 2001). However, most more recent isolates investigated were isolated from broiler flocks.

Since the 1960s, IBV has been controlled by vaccination. However, the vaccines still in use today were designed to prevent the nephritis (uraemia) that had been such a commercial problem during the 1940s-1960s. At the present time, the main effect of IBV on broiler flocks is respiratory disease whereas it appears that the main effects on layer flocks are reduced production (“egg drop”) and reductions in egg quality (Cook and Huggins, 1986, Chubb, 1987). Therefore, it is relevant to investigate the suitability of the current vaccines in protecting birds against challenge in the field.

The current project investigated the use of two strains of IBV vaccine virus, VicS and A3, first vaccination at either day-old or two weeks, administration of vaccine by eyedrop, coarse spray or in water, regular revaccination during lay and the level of protection of birds not vaccinated beyond 14 weeks of age against challenge with T-strain IBV.

1. Preliminary study: effect of age at first vaccination and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) in cockerels

1.1 Introduction

There has been some discussion concerning the optimal age for first vaccination against IBV. Although vaccination at day-old by coarse spray in the hatchery is the most convenient and commercially practical way of administering the first vaccination, there is some evidence that 2 weeks of age is a more appropriate age. The presence of maternal antibody may render day-old vaccination less effective than vaccination at 2 weeks. In addition, route of vaccination may influence vaccination effectiveness. Eyedrop is rarely used in the commercial industry where either coarse spray or in water vaccination is the norm. However, eyedrop ensures that the vaccinated birds receive a full dose of vaccine and is therefore used in experimental situations.

This Preliminary Study was conducted in cockerels to evaluate the use of a single strain of vaccine (VicS), two different ages at first vaccination (day-old and two weeks) and three different routes of vaccination (eye drop, coarse spray and in water).

1.2 Objectives

The aim of this experiment was to investigate the effect of age at first vaccination and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) in cockerels, in order to optimise infectious bronchitis vaccination protocols for laying hens. In addition, it was designed to test the ability of the researchers to maintain birds free from IBV in the experimental isolation facilities at the University of New England and to test the challenge virus (T-strain IBV).

1.3 Methodology

1.3.1 Animals and Vaccination Treatments

Day-old ISA Brown cockerels (350) were purchased from the Winton Hatchery near Tamworth, NSW and transferred to isolation pens at the University of New England, Armidale, NSW. The birds were reared according to standard commercial practice and fed starter diet from 1day old to 6 wks, followed by grower diet. There were seven experimental groups, each of 50 birds, as shown in Table 1.1. For all vaccinated groups, the vaccine used was Webster's VicS strain IBV (Fort Dodge).

Table 1.1 Experimental Treatment Groups

Group Abbreviation	Age at First Vaccination	Route of Vaccination
Control	No vaccination	No vaccination
Eyedrop I	Day-old	Eyedrop
Spray I	Day-old	Coarse Spray
Water I	Day-old	In drinking water
Eyedrop II	Two weeks	Eyedrop
Spray II	Two weeks	Coarse Spray
Water II	Two weeks	In drinking water

1.3.2 Blood Samples and Kidney Samples

Blood samples were taken from ten birds from each group at 7 weeks of age and birds were then re-vaccinated at 8 weeks, by the same routes as at day old or two weeks (except for the Control Group which was not re-vaccinated). A second series of blood samples was taken from ten birds per group at 11 weeks of age and some birds were euthanased for determination of body weight and kidney weights at 9, 10 and 11 weeks of age.

Fifteen birds from each group were transferred to a poultry isolation shed at 11 weeks of age and exposed to T strain IBV (10^4 CD_{50} per bird) to test for resistance provided by vaccination. The challenge virus was purchased from Dr. Jagoda Ignjatovic of the CSIRO Australian Animal Health Laboratory in Geelong. One week later (12 weeks of age), birds from all groups were euthanased and body weights and kidney weights measured. Further blood samples were taken from control birds only at 13 and 14 weeks of age and some birds were euthanased at each of these ages for determination of body weight and kidney weights. Clinical symptoms, wet droppings and mortality were observed in the unvaccinated control birds after exposure to T strain IBV. Antibody titres were determined by ELISA (KPL ProFLOK IBV ELISA kit, Birling Avian Laboratories, Bringelly, NSW).

1.3.3 Kidney Histology

The right and left kidneys of the birds that were euthanased were fixed by immersion in 10 percent neutral buffered formalin. Tissue from birds in each treatment and age group with the largest and smallest kidneys (as a percentage of body weight) were prepared for histological examination. Slices of the kidneys, 5 mm thick, were cut at right angles to the long axis approximately half way along each kidney division. Sections were embedded in paraffin wax, sectioned at a thickness of 5μm and stained with haematoxylin and eosin. Stained sections were observed under a light microscope. The numbers of kidneys observed were:

- Normal kidneys: two birds, two kidneys per bird, three divisions per kidney (12 blocks of tissue);
- Before exposure to T-strain IBV: six birds per treatment group, two kidneys per bird, one division per kidney (84 blocks of tissue);
- Unvaccinated group: one bird that died in addition to collections of six birds at each of the sample times (1, 2 and 3 weeks), two kidneys per bird, three divisions per kidney (114 blocks of tissue);
- Vaccinated group: two birds for all groups, two kidneys per bird, three divisions per kidney; plus the cranial division of both kidneys for an additional three birds in the three groups that showed histopathology (18 blocks of tissue).

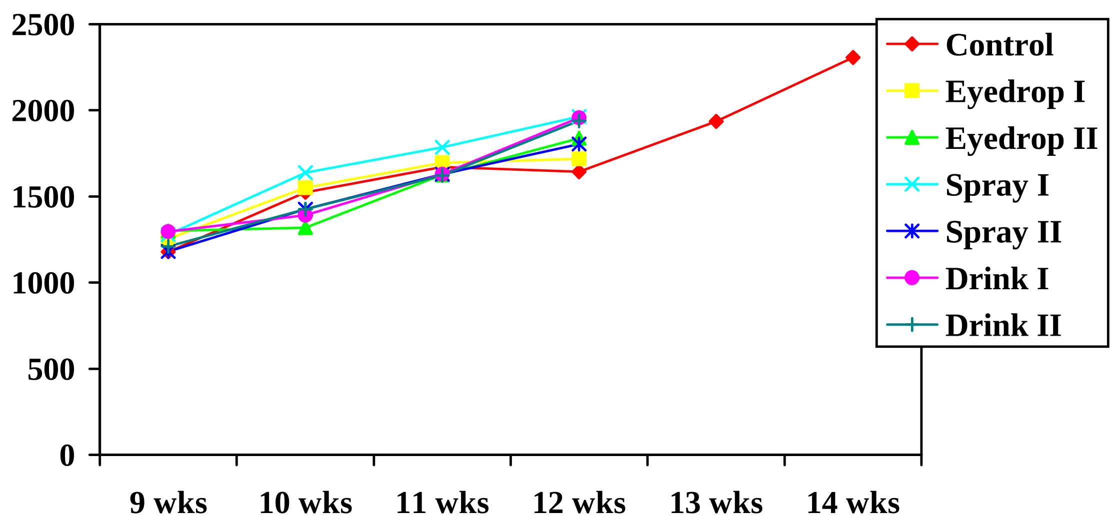
1.3.4 Presentation of Data
Data are presented in the form of tables and graphs. For most of the graphs, tables containing the data are contained in Appendix A at the end of the report.

1.3.5 Statistical Analysis
Analysis of Variance (ANOVA) was used to test the effect of the age and routes of vaccination on body weight, and total, right, and left kidney weight as a percentage of body weight. Fisher's protected LSD was utilized to separate means when significant main effects were observed (Steel and Torrie, 1980). All data were analysed using STATVIEW software. Statistical significance was assumed at $P<0.05$.

1.4 Detailed Results

1.4.1 Body Weight
There was little effect of age and routes of vaccination on bird body weight (Figure 1.1 and Table A1). At 12 weeks of age, body weight was slightly lower in the control group.

Figure 1.1 Body Weight (BW) of Vaccinated and Control Birds (g).



1.4.2 Kidney Weights
The weights of the right and left kidneys of the birds are shown in Tables 1.2 and 1.3, respectively. Total kidney weights are shown in Table 1.4. One week following exposure to T-strain IBV, kidney weight was highest in the control birds.

Table 1.2 Right kidney weight (g) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages
Control	5.52^{C} ±0.3	6.41^{BC} ±0.4	6.71^{B} ±0.2	9.00^{A} ±1.0	8.24^{A} ±0.3	9.39^{A} ±0.2	6.91^{a} ±0.3
Eyedrop at day-old	6.42 ±0.5	5.94 ±0.5	6.58 ±0.1	6.54 ±0.7			6.49^{a} ±0.1
Eyedrop at 2 weeks	6.45 ±0.4	5.69 ±0.4	6.22 ±0.2	7.83 ±0.4			6.40^{a} ±0.2
Coarse spray at day-old	5.87 ±0.5	6.54 ±0.2	6.84 ±0.3	7.82 ±0.5			6.84^{a} ±0.2
Coarse spray at 2 weeks	5.19 ±0.3	5.64 ±0.3	6.48 ±0.2	7.94 ±0.4			6.45^{a} ±0.2
In water at day-old	6.13 ±0.4	5.88 ±0.3	5.88 ±0.2	8.23 ±0.2			6.16^{a} ±0.2
In water at 2 weeks	5.85 ±0.3	6.53 ±0.2	6.54 ±0.2	8.44 ±0.1			6.71^{a} ±0.2
All groups	5.92^{c} ±0.2	$6.10b^{c}$ ±0.1	6.43^{b} ±0.1	7.97^{a} ±0.2			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
No significant effect of Treatment Group
Significant interaction between stage and treatment group ($P=0.0407$)
For control only, significant effect of stage of experiment (weeks) ($P<0.0001$)

Table 1.3 Left kidney weight (g) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages
Control	5.76^{D} ±0.41	7.41^{CD} ±0.39	6.49^{D} ±0.16	9.03^{AB} ±1.17	8.33^{BC} ±0.49	10.39^{A} ±0.63	6.97^{a} ±0.30
Eyedrop at day-old	6.22 ±0.33	6.30 ±0.43	6.69 ±0.16	6.88 ±0.56			6.63^{a} ±0.14
Eyedrop at 2 weeks	6.08 ±0.52	5.63 ±0.36	6.34 ±0.16	7.61 ±0.47			6.40^{a} ±0.15
Coarse spray at day-old	5.72 ±0.48	6.75 ±0.14	7.06 ±0.29	7.43 ±0.37			6.94^{a} ±0.21
Coarse spray at 2 weeks	5.13 ±0.29	6.00 ±0.54	6.54 ±0.23	8.29 ±0.49			6.56^{a} ±0.21
In water at day-old	5.25 ±0.29	5.79 ±0.19	5.97 ±0.19	8.05 ±0.29			6.12^{a} ±0.18
In water at 2 weeks	5.63 ±0.06	6.38 ±0.27	6.62 ±0.19	8.74 ±0.27			6.77^{a} ±0.19
All groups	5.69^{a} ±0.14	6.32^{b} ±0.16	6.51^{b} ±0.08	8.01^{a} ±0.4			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
No significant effect of treatment group
No interaction between stage and treatment group
For control only, significant effect of stage of experiment (weeks) ($P<0.0001$)

Table 1.4 Total kidney weight (g) of cockerels exposed to T-strain IBV following different vaccination protocols

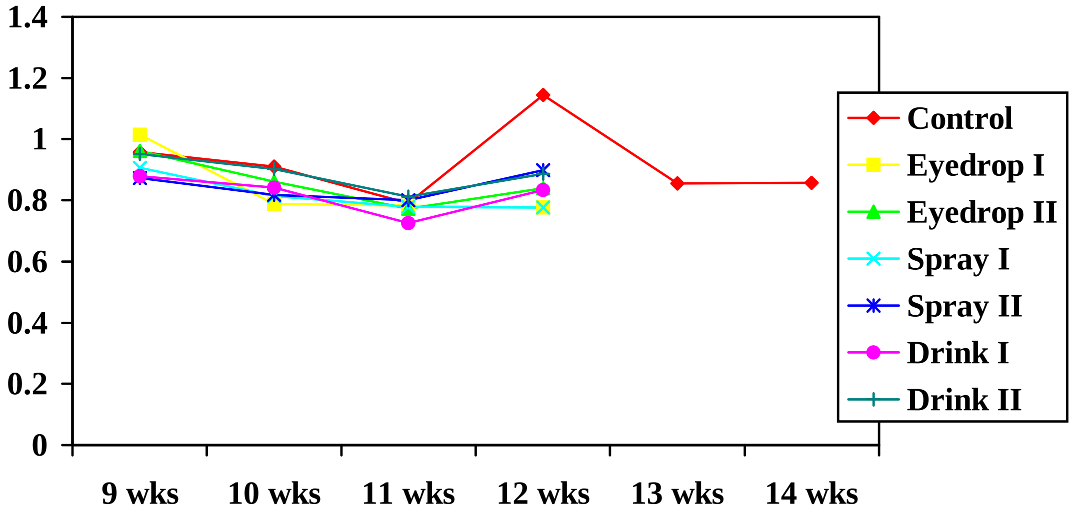
	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages (9-12)
Control	11.27^{D} ±0.7	13.83^{CD} ±0.7	13.20^{D} ±0.3	18.03^{AB} ±2.2	16.57^{BC} ±0.8	19.78^{A} ±0.8	13.88^{a} ±0.6
Eyedrop at day-old	12.65 ±0.8	12.24 ±0.7	13.27 ±0.2	13.42 ±1.2			13.12^{a} ±0.2
Eyedrop at 2 weeks	12.54 ±0.9	11.33 ±0.7	12.56 ±0.3	15.45 ±0.8			12.80^{a} ±0.3
Coarse spray at day-old	11.60 ±0.7	13.29 ±0.3	13.90 ±0.3	815.25 ±0.9			13.77^{a} ±0.3
Coarse spray at 2 weeks	10.33 ±0.6	11.64 ±0.8	13.02 ±0.4	16.23 ±0.9			13.01^{a} ±0.4
In water at day-old	11.39 ±0.6	11.67 ±0.4	11.85 ±0.4	16.28 ±0.4			12.28^{a} ±0.3
In water at 2 weeks	11.47 ±0.2	12.91 ±0.4	13.16 ±0.4	17.18 ±0.3			13.48^{a} ±0.3
All groups	11.61^{c} ±0.3	12.41b^{c} ±0.3	12.94^{b} ±0.1	15.98^{a} ±0.4			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
No significant effect of treatment group
Significant interaction between stage and treatment group ($P=0.0150$)
For control only, significant effect of stage of experiment (weeks) ($P<0.0001$)

1.4.3 Total Kidney Weight as a Percentage of Body Weight

When birds were 9, 10 and 11 weeks of age, prior to the exposure of birds to T strain IBV, there were no differences among the groups for total kidney weight as a percentage of body weight. One week after the exposure to T strain IBV, kidney weights as a percentage of body weight were unchanged in all the vaccinated groups of birds, whereas kidneys of the control group were significantly enlarged. However, at two and three weeks following exposure to T strain IBV, the kidney weight to body weight ratios of the control group had returned to normal (Figure 1.2, Table A2).

Figure 1.2 Total Kidney Weight as a Percentage of Body Weight for Vaccinated and Control Birds (%)



1.4.4 Right and Left Kidney Weight as Percentages of Body Weight

Consistent with the results of total kidney weight, when birds were 9, 10 and 11 weeks of age, prior to the exposure of birds to T strain IBV, there were no differences among the groups for the weights of right or left kidneys as a percentage of body weight. One week after the exposure to T strain IBV (at 12 weeks), kidney weights as a percentage of body weight were unchanged in all the vaccinated groups of birds, whereas both right and left kidneys of the control group were significantly enlarged and bigger than the other groups. However, at two and three weeks following exposure to T strain IBV, both the right and left kidney to body weight ratios of the control group had returned to normal (Figures 1.3 and 1.4; Tables A3 and A4).

Figure 1.3 Right Kidney Weight as a Percentage of Body Weight for Vaccinated and Control Birds (%)

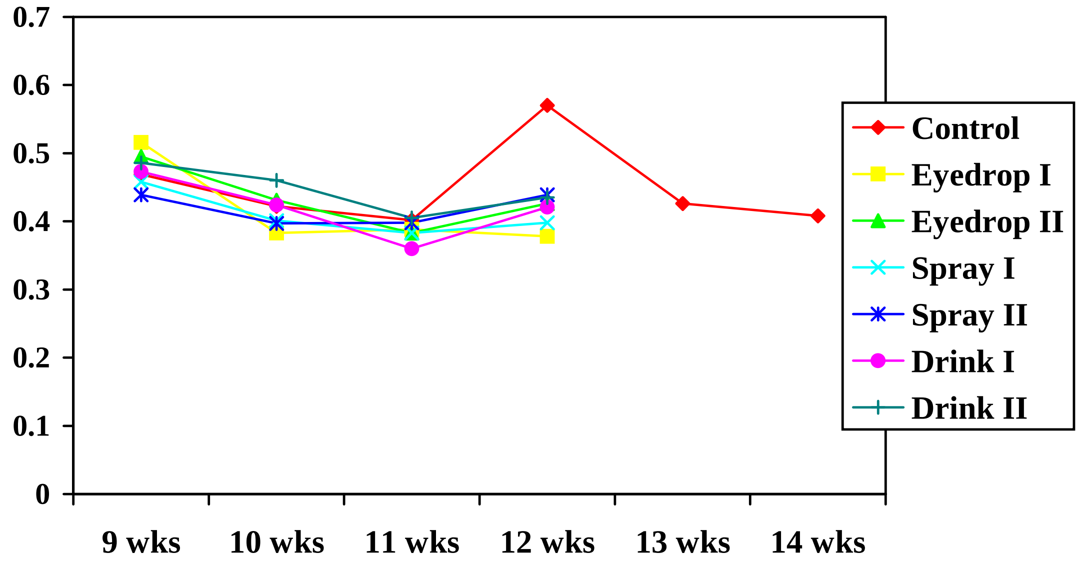
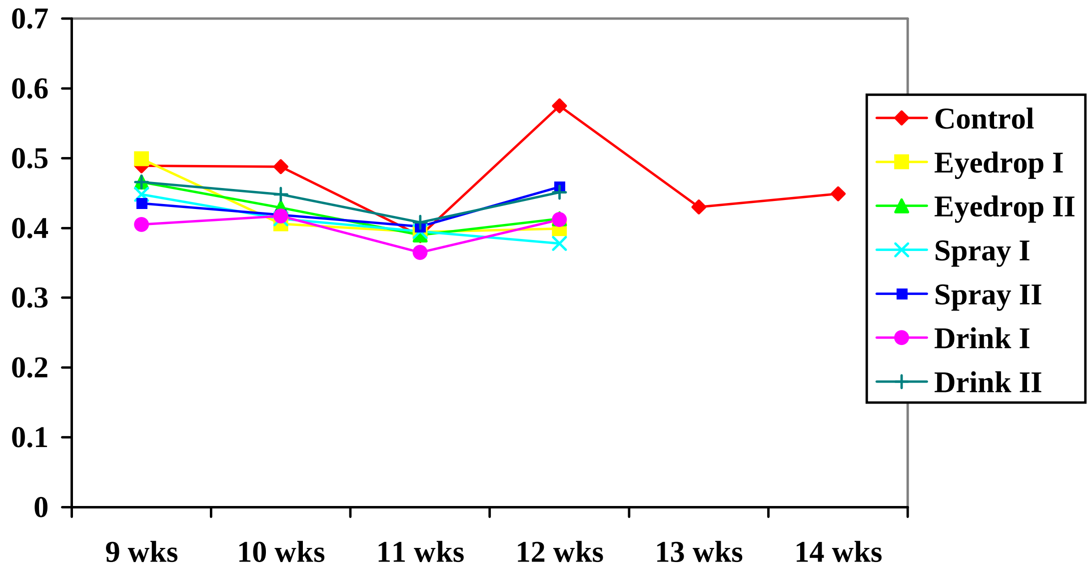


Figure 1.4 Left Kidney Weight as a Percentage of Body Weight for Vaccinated and Control Birds (%)



1.4.5 Heavy : Light Ratios

The ratios of the heavier to the lighter kidney for each bird were calculated and are shown in Table 1.5. There were no significant differences in size between the right and left kidneys and no evidence of kidney asymmetry.

Table 1.5 Heavy/light kidney ratio of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages
Control	1.05 ±0.01	1.17 ±0.08	1.07 ±0.01	1.07 ±0.03	1.04 ±0.01	1.10 ±0.05	1.08 ±0.01
Eyedrop at day-old	1.11 ±0.02	1.11 ±0.09	1.09 ±0.03	1.07 ±0.03			1.09 ±0.02
Eyedrop at 2 weeks	1.07 ±0.05	1.09 ±0.04	1.09 ±0.01	1.05 ±0.02			1.08 ±0.01
Coarse spray at day-old	1.18 ±0.09	1.03 ±0.01	1.23 ±0.14	1.07 ±0.03			1.19 ±0.09
Coarse spray at 2 weeks	1.07 ±0.02	1.12 ±0.05	1.14 ±0.14	1.04 ±0.02			1.11 ±0.02
In water at day-old	1.17 ±0.08	1.07 ±0.03	1.09 ±0.01	1.06 ±0.03			1.09 ±0.01
In water at 2 weeks	1.10 ±0.03	1.05 ±0.03	1.07 ±0.01	1.07 ±0.02			1.07 ±0.01
All groups	1.11 ±0.02	1.09 ±0.02	1.11 ±0.02	1.06 ±0.01			

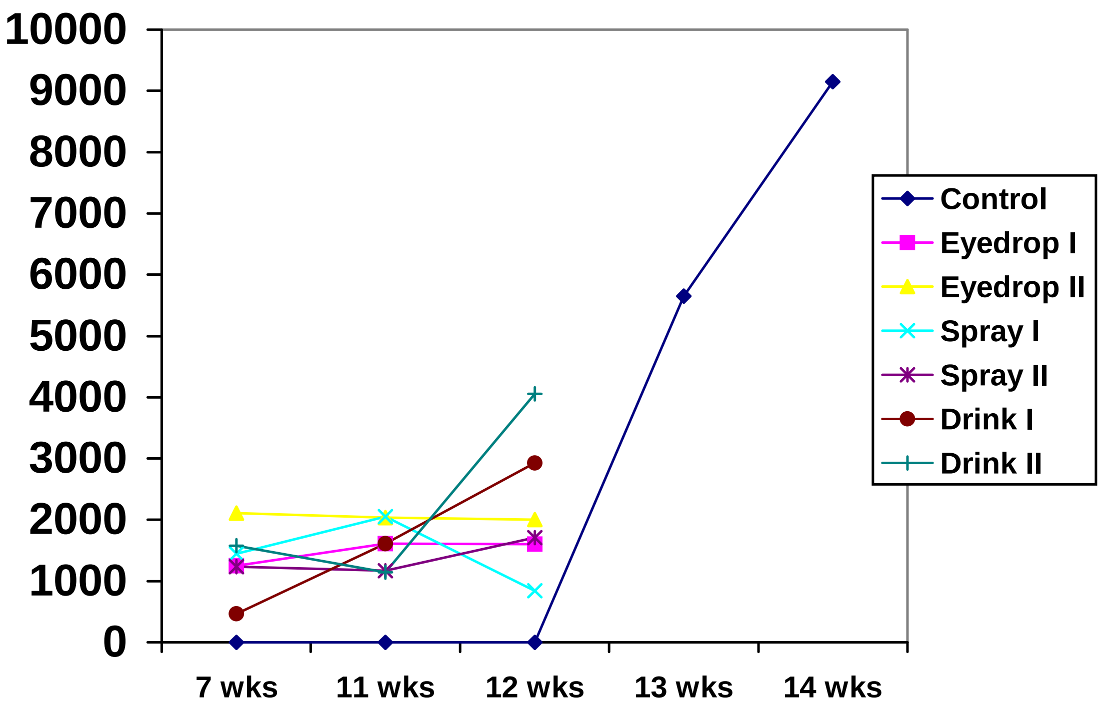
Values are Mean ± S.E.
No significant differences among ages (weeks) and treatments

1.4.6 IB Antibody Titres and Clinical Symptoms

There was variation among the titre levels for the vaccinated groups although there were no obvious differences based on age at vaccination or route of administration of vaccine. The mean titre for all vaccinated groups, which offered protection from exposure to T strain IBV, was 1476. In the unvaccinated control group, antibody titres remained at zero levels before exposure to T strain IBV. However, the titre levels increased at two weeks following exposure (5647), with a further increase at three weeks (9153), as shown in Figure 1.5 (and Table A6). It is worth noting that the IBV antibody titres were not noticeably elevated at one week following challenge.

Concomitant with increased IB antibody titres, the exposure caused respiratory symptoms, wet droppings and mortality in unvaccinated control birds. One of the unvaccinated birds died one week following exposure to T-strain IBV.

Figure 1.5 IB Antibody Titres of Vaccinated and Control Birds



1.4.7 Plasma Electrolytes

Haematocrit (also known as packed cell volume) varied significantly with age ($P<0.0001$), being lowest at 7 weeks of age. There were also significant differences among the treatment groups ($P=0.0001$) (Table 1.6). Plasma concentrations of sodium (Table 1.7), potassium (Table 1.8) and chloride (Table 1.9) were not statistically significantly different among the vaccination treatment groups. However, plasma potassium and chloride showed some differences with bird age.

Table 1.6 Haematocrit (%) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 7	Week 11	Week 12	Week 13	Week 14	All ages
Control	31.30 ^{B} ± 0.4	34.5 ^{A} ± 0.5	33.55 ^{A} ± 0.7	34.28 ^{A} ± 0.7	34.86 ^{A} ± 0.5	33.12^{a} ±0.4
Eyedrop at day-old	29.72 ±0.7	33.90 ±0.7	32.35 ±0.7			32.07^{a} ±0.5
Eyedrop at 2 weeks	29.25 ±0.1	31.65 ±1.0	31.80 ±0.5			30.90^{c} ±0.5
Coarse spray at day-old	31.65 ±0.5	34.25 ±0.5	33.2 ±0.7			33.03^{ab} ±0.4
Coarse spray at 2 weeks	32.4 ±0.5	31.95 ±0.9	31.40 ±0.7			31.92^{bc} ±0.4
In water at day-old	30.9 ±0.6	32.55 ±0.6	31.30 ±0.4			31.58^{bc} ±0.3
In water at 2 weeks	33.0 ±0.57	33.95 ±0.8	32.05 ±0.6			33.00^{ab} ±0.4
All groups	31.19^{c} ±0.3	33.25^{a} ±0.3	32.24^{b} ±0.2			

Values are Mean ± S.E.
$^{A\text{-}B}$ For the control group, values with different superscripts are significantly different
$^{a\text{-}c}$ Significant effect of stage of experiment (weeks) (P<0.0001)
$^{a\text{-}c}$ Significant effect of treatment group (P=0.0001)
Significant interaction between stage and treatment group (P=0.0322)

Table 1.7 Plasma sodium (mmol/L) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 7	Week 11	Week 12	Week 13	Week 14	All ages
Control	143.98 ±3.2	151.35 ±6.1	147.82 ±5.1	146.29 ±3.2	158.15 ±11.7	147.72 ±2.8
Eyedrop at day-old	144.39 ±6.0	144.26 ±10.3	152.23 ±5.5			146.96 ±4.3
Eyedrop at 2 weeks	157.75 ±7.6	149.31 ±9.1	163.39 ±6.4			156.82 ±4.5
Coarse spray at day-old	167.41 ±4.3	140.37 ±6.2	150.51 ±5.3			152.76 ±3.6
Coarse spray at 2 weeks	164.44 ±6.0	154.67 ±10.7	151.77 ±5.5			156.96 ±4.4
In water at day-old	151.49 ±4.2	153.21 ±4.9	140.82 ±5.2			148.51 ±2.8
In water at 2 weeks	152.84 ±5.9	149.27 ±8.1	156.01 ±4.9			152.71 ±3.6
All groups	154.61 ±2.2	148.92 ±3.0	151.79 ±2.1			

Values are Mean ± S.E.
No significant effects of stage or treatment group

Table 1.8 Plasma potassium (mmol/L) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 7	Week 11	Week 12	Week 13	Week 14	All ages
Control	4.27^{C} ±0.1	5.10^{B} ±0.2	5.32^{B} ±0.2	5.49^{AB} ±0.2	6.13^{A} ±0.5	4.89 ±0.1
Eyedrop at day-old	3.05 ±0.1	4.96 ±0.3	5.83 ±0.4			4.62 ±0.3
Eyedrop at 2 weeks	3.72 ±0.2	4.83 ±0.3	5.79 ±0.3			4.78 ±0.2
Coarse spray at day-old	3.70 ±0.1	4.62 ±0.2	5.93 ±0.2			4.75 ±0.2
Coarse spray at 2 weeks	3.97 ±0.2	5.24 ±0.4	5.85 ±0.2			5.02 ±0.2
In water at day-old	3.70 ±0.1	4.58 ±0.2	5.25 ±0.2			4.51 ±0.2
In water at 2 weeks	4.38 ±0.1	3.93 ±0.2	5.52 ±0.3			4.61 ±0.2
All groups	3.83^{c} ±0.1	4.75^{b} ±0.1	5.64^{a} ±0.1			

Values are Mean ± S.E.
$^{A-B}$ For the control group, values with different superscripts are significantly different
$^{a-c}$ Significant effect of stage of experiment (weeks) (P<0.0001)
No significant effect of Treatment Group
Significant interaction between stage and treatment group (P=0.0003)

Table 1.9 Plasma chloride (mmol/L) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 7	Week 11	Week 12	Week 13	Week 14	All ages (7-14 wks)
Control	116.60 ±4.5	123.60 ±5.4	113.60 ±4.6	110.3 ±4.0	117.00 ±5.2	117.93 ±2.8
Eyedrop at day-old	113.70 ±3.8	140.30 ±8.5	122.20 ±5.7			125.40 ±4.1
Eyedrop at 2 weeks	125.90 ±7.1	141.20 ±6.0	124.20 ±6.5			130.33 ±3.9
Coarse spray at day-old	133 ±4.2	127.50 ±7.0	119.00 ±2.8			126.50 ±3.0
Coarse spray at 2 weeks	125.50 ±3.7	147.40 ±10.1	115.10 ±4.3			129.33 ±4.5
In water at day-old	126.20 ±4.5	132.90 ±5.8	112.30 ±5.6			123.80 ±3.4
In water at 2 weeks	127.30 ±5.5	130.20 ±6.6	122.30 ±5.6			126.60 ±3.4
All groups	124.03^{b} ±1.9	134.73^{a} ±2.8	118.38^{b} ±1.9			

Values are Mean ± S.E.
No significant effect of stage of experiment (weeks) for control group
$^{a-c}$ Significant effect of stage of experiment (weeks) (P<0.0001)
No significant effect of Treatment Group
No significant interaction between stage and treatment group

1.4.8 Kidney Histology

The unvaccinated birds, at one week post-infection, all showed some degree of histopathology. However, the severity of the lesions varied between individuals. The histopathological signs displayed by the birds in this group ranged from minimal monocyte infiltration, to dense widespread lymphocyte infiltration and necrosis of renal tubules. Incursion of both mononuclear and polynuclear white blood cells into the interstitial tissue of the kidneys was clearly observed mostly in the medullary region originating around collecting ducts. The collecting ducts in the most affected areas of the kidney were clearly distended and their epithelial cells elongated. Poly-nuclear cells with granules in the cytoplasm were present in the damaged collecting ducts. Although it varied between birds, generally the pathological signs were observed equally in all three sections of both the right and left kidney. Unvaccinated birds that were euthanased at two and three weeks after exposure to the T-strain virus showed a reduction in area of dense monocyte infiltration to discrete patches. An increasing incursion of pathology into cortical areas of the kidney was also evident. This suggests a progression of the nephritis commencing with lymphocyte infiltration first of the medullary (later the cortex), then continuing to include necrosis of collecting ducts and tubules.

1.5 Discussion and Conclusions

These results indicate that both ages at vaccination (day-old and two weeks of age), and all routes of vaccine administration (eyedrop, coarse spray and water vaccination) were effective in protecting birds against the effects of exposure to T strain IBV. Ratanasethakul and Cumming (1983) found that the route of administration of A3 vaccine virus influenced the degree of immunity that was produced.

The unvaccinated birds reacted to exposure to T strain IBV with respiratory symptoms, wet droppings and mortality as well as a significant enlargement of both kidneys, an indication of the nephropathogenic effects of T strain IBV in birds which are not protected by an adequate vaccination program. Butcher and Miles (2001) report that, world-wide, some strains of the virus, nephropathogenic IB viruses, infect the kidneys and cause permanent renal damage. Infected chickens produce watery droppings, resulting in wet litter. Urates are common and can be identified easily in the droppings and in the kidneys and ureters at necropsy. The kidneys of affected birds are pale, mottled, and can be 2 to 3 times their normal size. Even though mortality in uncomplicated IB outbreaks can be relatively low, infection with nephropathogenic strains may cause high mortality.

Based on histological examination, all of the vaccination protocols appear to have protected the birds to some degree. Individual birds in most treatments showed varying levels of minor monocyte infiltration but no further lesions, indicating a mild reaction that has been halted by the birds' immune systems. However, a reaction comparable in severity to those observed in the unvaccinated birds was seen in the groups that were first vaccinated by drinking water at day old or spray application at two weeks of age. These protocols would appear to have failed to protect the birds as well as the other methods. The lack of protection achieved by drinking water vaccination at day old may have been reasonably expected. The lack of protection of the kidneys, in the spray vaccinated birds was unusual and may be due to a number of reasons including exposure to a particularly high dose of challenge virus or the health of these birds being compromised in some other way prior to challenge.

The findings of the present study emphasise the importance of optimising vaccination protocols against infectious bronchitis virus in order to maintain maximum profitability of the industry.

2. Trial 1: effects of vaccine strain and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) in laying hens - Rearing Phase

2.1 Introduction

At the time that this experiment was conducted, the most common strains of IBV vaccine used by the Australian egg industry were Webster's VicS (Fort Dodge) and A3 (Fort Dodge). Some producers use only one strain (usually VicS) whereas others alternate the two strains during the rearing phase. Routes of vaccine administration vary with coarse spray at the hatchery and either coarse spray or in water vaccination for subsequent vaccinations being the norm. Some producers do not revaccinate for IBV once the birds have come into lay. However, increasingly, poultry veterinarians are recommending regular revaccination, usually every 8 weeks throughout lay.

2.2 Objectives

The first main trial of the project was designed to investigate the effects of vaccine strain, route of vaccination and regular revaccination on the performance of laying hens. This chapter covers the rearing phase to 16 weeks of age.

2.3 Methodology

Day-old ISA Brown hens (625) were purchased from the Winton Hatchery near Tamworth, NSW and transferred to isolation pens (geographically isolated in relation to natural wind directions) at the University of New England, Armidale, NSW. The birds were reared according to standard commercial practice. There were seven experimental groups, each of 89 birds: Control (No vaccination), VicS eye (VicS vaccine by eye drop at day old), VicS spray (VicS by coarse spray at day old), VicS water (VicS in water at day old), A3 eye (A3 vaccine strain by eye drop at day old), A3 spray (A3 by coarse spray at day old), A3 water (A3 in water at day old). Blood samples were taken from ten birds from each group at 4 weeks of age and birds were then revaccinated with the opposite strain of vaccine to that used at day-old, via the same routes as day old. The Control Group remained unvaccinated.

Blood samples were taken from ten birds per group at 6 weeks of age. Birds were vaccinated for AE (avian encephalomyelitis) at 12 weeks of age. At 14 weeks of age, all birds (including the Control birds) were revaccinated with VicS vaccine strain by eye drop. At 15 weeks of age, all birds were transferred to two poultry isolation sheds equipped with 3-bird commercial-style cages. Blood samples were taken at 16 weeks.

Analysis of Variance was used to test the effect of vaccination treatment and regular revaccination on each measured parameter. Fisher's protected LSD was utilized to separate means when significant effects were observed. Statements of statistical significance were based on $P<0.05$.

2.4 Detailed Results

As shown in Figure 2.1 and Table B1, body weight was very similar among the different vaccination treatment groups.

For blood measurements, there were technical problems with the measurement of haematocrit at week 16. Therefore, Table 2.1 shows haematocrit for weeks 4 and 6 only. Haematocrit remained constant across the two week period. For plasma sodium concentration, there was an overall increase ($P<0.0001$) to 16 weeks of age as shown in Table 2.2. The pattern was similar for plasma potassium ($P<0.0001$ - Table 2.3) and plasma ionised calcium ($P<0.0001$ - Table 2.4). For potassium, there was a significant interaction ($P=0.0019$) between bird age and treatment group.

During the rearing phase, the IBV antibody titres increased significantly ($P<0.0001$) from a mean for all treatment groups of 276.9 at 4 weeks of age to means of 1733.8 and 1613.6 at 6 and 16 weeks of age, respectively (Table 2.5 and Figure 2.2). There was also a significant difference ($P=0.0250$) among the treatment groups with titres being highest for A3 vaccine administered by eye drop and lowest for the control. The control group remained negative for IBV until after the first vaccination of this group at 14 weeks of age.

Table 2.1 Haematocrit value (%) of ISA brown hens exposed to different IB vaccination protocols in rearing phase

	Week 4	Week 6	Both Ages
Control	29.6 ±0.5	28.7 ±0.6	29.1 ±0.4
VicS, eyedrop at day-old	29.6 ±0.6	29.6 ±0.6	29.6 ±0.4
VicS, coarse spray at day-old	30.1 ±0.8	29.1 ±0.4	29.6 ±0.5
VicS, in water at day-old	27.7 ±0.7	28.4 ±0.6	28.0 ±0.45
A3, eyedrop at day-old	29.2 ±0.4	28.7 ±0.8	28.9 ±0.4
A3, coarse spray at day-old	29.0 ±0.4	29.8 ±0.4	29.4 ±0.3
A3, in water at day-old	28.6 ±0.4	29.1 ±0.5	28.8 ±0.3
All Groups	29.1 ±0.2	29.0 ±0.2	

Values are Mean ± S.E.

Figure 2.1 Body weight of the seven vaccination treatment groups during the rearing phase

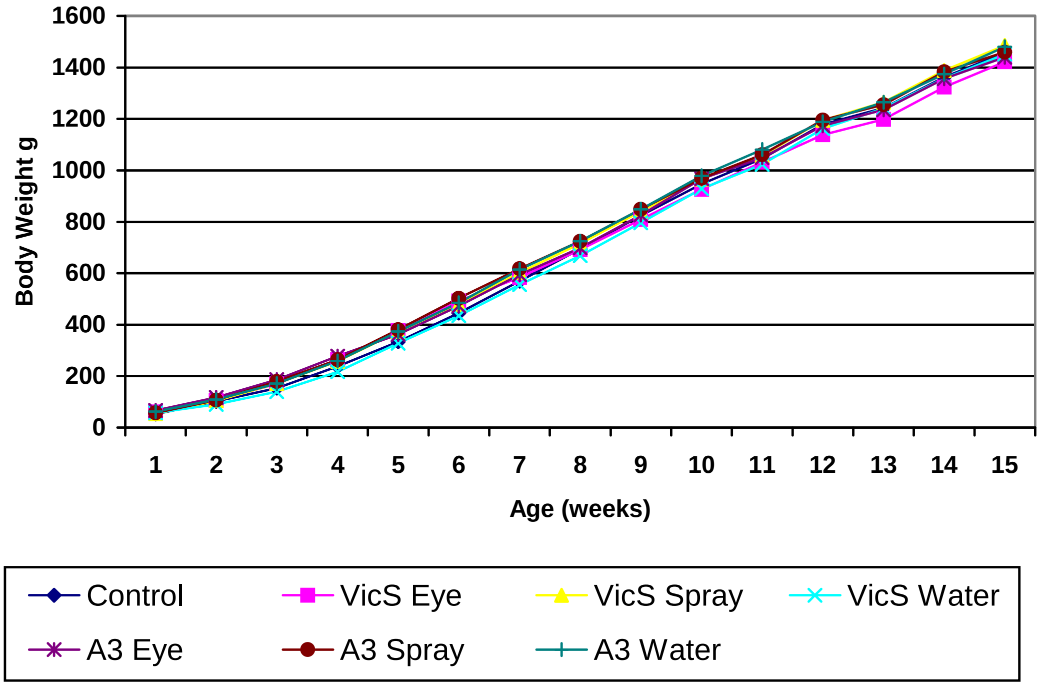


Table 2.2. Plasma sodium (Na^+, mmol/L) of ISA brown hens exposed to different IB vaccination protocols in rearing phase

	Week 4	Week 6	Week 16	All Ages
Control	136.94 ±1.4	135.35 ±1.4	146.57 ±1.1	139.62 ±1.2
VicS, eyedrop at day-old	137.81 ±1.0	134.38 ±2.1	145.55 ±0.7	139.25 ±1.2
VicS, coarse spray at day-old	138.37 ±0.7	133.83 ±1.8	147.78 ±0.8	139.99 ±1.3
VicS, in water at day-old	136.46 ±1.9	134.47 ±1.7	145.36 ±1.3	138.76 ±1.3
A3, eyedrop at day-old	136.39 ±2.1	136.25 ±1.5	146.63 ±0.5	139.76 ±1.2
A3, coarse spray at day-old	137.64 ±1.6	137.52 ±0.9	146.36 ±0.7	140.51 ±1.0
A3, in water at day-old	136.83 ±1.6	136.84 ±0.5	147.90 ±0.8	140.52 ±1.1
All Groups	137.21[b] ±0.6	135.52[c] ±0.6	146.59[a] ±0.3	

Values are Mean ± S.E.
Significant differences among ages (P<0.0001)

Table 2.3. Plasma potassium (K^+, mmol/L) of ISA brown hens exposed to different IB vaccination protocols in rearing phase

	Week 4	Week 6	Week 16	All Ages
Control	4.94 ±0.1	5.60 ±0.1	5.53 ±0.1	5.36 ±0.1
VicS, eyedrop at day-old	4.81 ±0.1	5.34 ±0.1	5.64 ±0.1	5.26 ±0.1
VicS, coarse spray at day-old	4.88 ±0.1	5.35 ±0.1	6.03 ±0.2	5.42 ±0.1
VicS, in water at day-old	4.56 ±0.1	5.39 ±0.1	5.93 ±0.1	5.29 ±0.1
A3, eyedrop at day-old	4.64 ±0.1	5.23 ±0.1	5.76 ±0.1	5.21 ±0.1
A3, coarse spray at day-old	4.51 ±0.1	5.32 ±0.1	6.00 ±0.1	5.28 ±0.1
A3, in water at day-old	4.53 ±0.1	5.48 ±0.1	5.95 ±0.1	5.32 ±0.1
All Groups	4.70[c] ±0.04	5.39[b] ±0.04	5.84[a] ±0.05	

Values are Mean ± S.E.
[a-c] Significant differences among ages (P<0.0001)
No significant difference among treatment groups
Significant interaction between age and treatment (0.0019)

Table 2.4 Plasma ionised calcium (Ca^{++} mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols in rearing phase

	Week 4	Week 6	Week 16	All Ages
Control	1.27 ±0.05	1.22 ±0.02	1.49 ±0.04	1.33 ±0.03
VicS, eyedrop at day-old	1.28 ±0.04	1.23 ±0.06	1.40 ±0.02	1.30 ±0.03
VicS, coarse spray at day-old	1.33 ±0.03	1.22 ±0.04	1.62 ±0.06	1.39 ±0.04
VicS, in water at day-old	1.22 ±0.06	1.28 ±0.02	1.43 ±0.05	1.31 ±0.03
A3, eyedrop at day-old	1.25 ±0.07	1.27 ±0.02	1.47 ±0.04	1.33 ±0.03
A3, coarse spray at day-old	1.30 ±0.03	1.30 ±0.03	1.48 ±0.05	1.36 ±0.03
A3, in water at day-old	1.24 ±0.06	1.28 ±0.03	1.51 ±0.03	1.34 ±0.03
All Groups	1.27[b] ±0.02	1.26[b] ±0.01	1.49[a] ±0.02	

Values are Mean ± S.E.
[a-c] Significant differences among ages (P<0.0001)
No significant difference among treatment groups

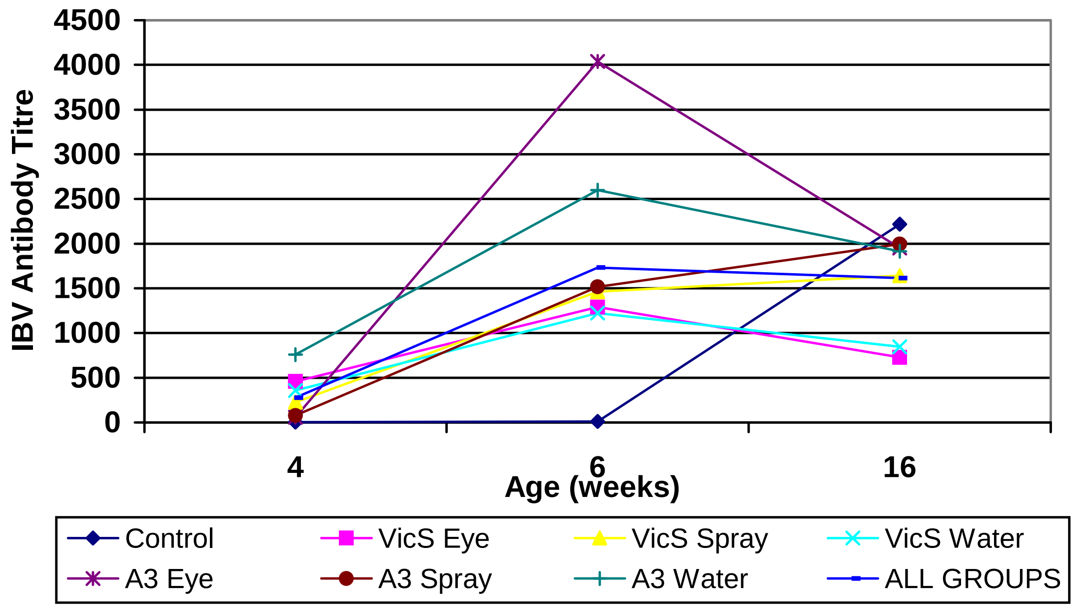
Table 2.5 IBV antibody titres of ISA brown hens exposed to the following different IB vaccination protocols in rearing phase

	Week 4	Week 6	Week 16	All Ages
Control	5.00 ±2.7	8.60 ±4.3	2216.30 ±616.5	743.30^{C} ±277.0
VicS, eyedrop at day-old	457.90 ±295.9	1291.70 ±528.1	727.50 ±312.6	825.7^{C} ±228.4
VicS, coarse spray at day-old	226.10 ±96.3	1463.30 ±416.38	1642.20 ±530.7	1110.53^{BC} ±248.4
VicS, in water at day-old	357.60 ±128.8	1222.80 ±464.1	845.10 ±432.0	808.50^{C} ±218.3
A3, eyedrop at day-old	57.90 ±33.3	4036.90 ±891.7	1952.60 ±494.1	2015.80^{A} ±445.8
A3, coarse spray at day-old	76.40 ±38.0	1515.20 ±485.5	1996.20 ±718.4	1195.93^{ABC} ±317.6
A3, in water at day-old	757.30 ±352.4	2597.90 ±816.5	1915.10 ±895.2	1756.77^{AB} ±429.7
All Groups	276.87^{B} ±73.3	1733.77^{A} ±252.9	1613.57^{A} ±226.0	

Values are Mean ± S.E.

Significant differences among treatments (0.0250), ages (<0.0001); interaction between ages and treatment (0.0009)

Figure 2.2 IBV antibody titres of the seven vaccination treatment groups during the rearing phase



2.5 Discussion of Results

The initial vaccination treatment given to the birds appeared to have little effect on body weight haematocrit or plasma electrolytes during the rearing phase. There was no evidence that either vaccine strain, VicS or A3, caused a “set-back” effect. The changes in haematocrit and plasma electrolytes appear to be related to the growth and development of the birds.

The control group of birds remained negative for IBV antibodies until after they were vaccinated with VicS at 14 weeks of age.

3. Trial 1: effects of vaccine strain and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) in laying hens - Laying Phase

3.1 Introduction

The results reported in this Chapter are for the laying phase of the same flock as described in Chapter 2 from 16-56 weeks of age.

3.2 Objectives

The objectives of this experiment were:

- To determine the production performance of birds that had received different initial vaccination protocols for IBV during the rearing phase
- To determine the effects on production performance of regular revaccination every 8 weeks during lay, as compared with no vaccination once the birds had come into lay
- To determine the effects of regular revaccination (versus no revaccination during lay) on IBV antibody titres

3.3 Methodology

The flock was reared as described in Chapter 2. At 14 weeks of age, all birds (including the Control birds which had not been vaccinated previously) were revaccinated with VicS vaccine strain by eye drop. At 15 weeks of age, all birds were transferred to two poultry isolation sheds equipped with 3-bird commercial-style cages. One-half of the birds from each treatment group were allocated to each shed, 2 birds per cage.

The birds in one shed were revaccinated every 8 weeks with VicS vaccine strain by coarse spray, whereas the birds in the other shed were not revaccinated beyond 14 weeks of age. For each group, body weight (BW) was recorded regularly.

Egg production, egg weight and the external appearance of the eggs were recorded daily. Faecal moisture was measured 1 and 2 weeks post revaccination. Every 4 weeks, 21 eggs of each group from each shed were collected for egg and egg shell quality measurements (a total of 294 eggs). Blood samples were taken from 5 birds from each group, in each shed, 3 weeks after revaccination for determination of the plasma electrolytes Na^+, K^+ and Ca^{++}, haematocrit, and antibody titres (Birling Avian Laboratories: IDEXX IBV ELISA kit). Clinical signs and mortality were recorded if observed and all mortalities autopsied.

Analysis of Variance was used to test the effect of vaccination treatment and regular revaccination on each measured parameter. Fisher's protected LSD was utilized to separate means when significant effects were observed. Statements of statistical significance were based on $P<0.05$.

3.4 Detailed Results

3.4.1 Body Weight and Feed Intake

There was no effect of vaccination treatment on the body weight of the birds. Body weights were within the target range recommended by the breeder company (Tables C1 and C2 in Appendix C). Feed intake measurement was made difficult by the fact that the birds were not beak-trimmed and tended to flick feed. Therefore, the values shown in Table C5 will be overestimates.

3.4.2 Hen-day Production

Hen-day production of non-revaccinated birds and revaccinated birds is shown in Figures 3.1 and 3.2 (also Tables C3 and C4 in Appendix C). As would be expected, there was a significant effect of hen age ($P<0.0001$) on egg production from 18 to 56 weeks of age, with production increasing to a peak of 94.5 eggs/100 hens/day at 29 weeks and then decreasing gradually to 83.8 eggs/100 hens/day at 56 weeks of age.

There was a small but statistically significant effect ($P<0.0001$) of regular revaccination on egg production between 18 and 56 weeks of age, with production during this period averaging 88.1 eggs/100 hens/day in the birds that were not revaccinated and 86.6 eggs/100 hens/day in the birds that were revaccinated regularly every 8 weeks from 22 weeks to 54 weeks (Table 3.1). This effect was particularly noticeable in early lay, 23-26 weeks, as shown in Figure 3.3. Initial vaccination treatment had a statistically significant effect ($P<0.0001$) on production from 18 to 56 weeks, as shown in Table 3.1. Production was slightly lower for the Control and A3 coarse spray initial vaccination treatment groups.

Table 3.1 Effect of initial vaccination treatment on production at 18-56 weeks (eggs/100 hens/day)

Group	Revaccinated regularly	Not revaccinated	Both treatments during lay
Control	84.3 ± 1.1	86.0 ± 1.2	^{b}85.1 ± 0.8
VicS, eyedrop at day-old	85.8 ± 1.2	89.3 ± 0.9	^{a}87.5 ± 0.8
VicS, coarse spray at day-old	86.2 ± 1.1	90.9 ± 0.9	^{a}88.5 ± 0.7
VicS, in water at day-old	87.4 ± 1.1	88.3 ± 1.0	^{a}87.8 ± 0.7
A3, eyedrop at day-old	88.5 ± 1.0	86.9 ± 1.2	^{a}87.7 ± 0.8
A3, coarse spray at day-old	86.9 ± 0.9	85.2 ± 1.2	^{b}86.0 ± 0.8
A3, in water at day-old	87.0 ± 1.0	90.2 ± 1.0	^{a}88.6 ± 0.7
All Treatment Groups	^{B}86.6 ± 0.4	^{A}88.1 ± 0.4	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Figure 3.1 Production (eggs/100 hens/day) from 16 to 56 weeks of age in birds that were not revaccinated regularly during lay

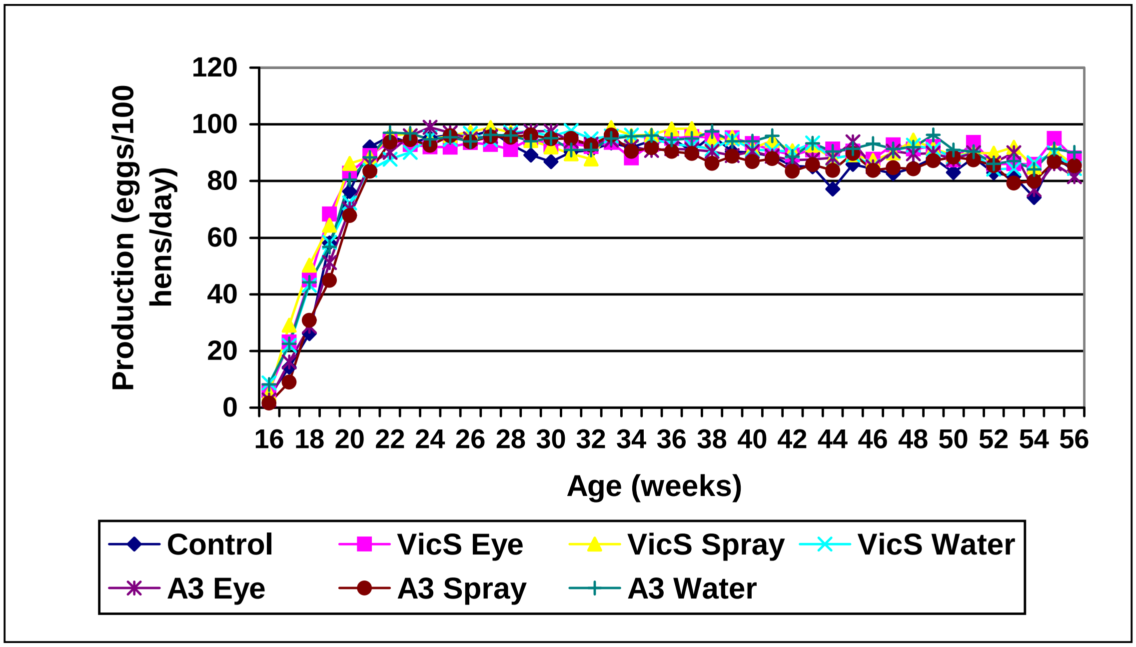


Figure 3.2 Production (eggs/100 hens/day) from 16 to 56 weeks of age in birds that were revaccinated regularly during lay

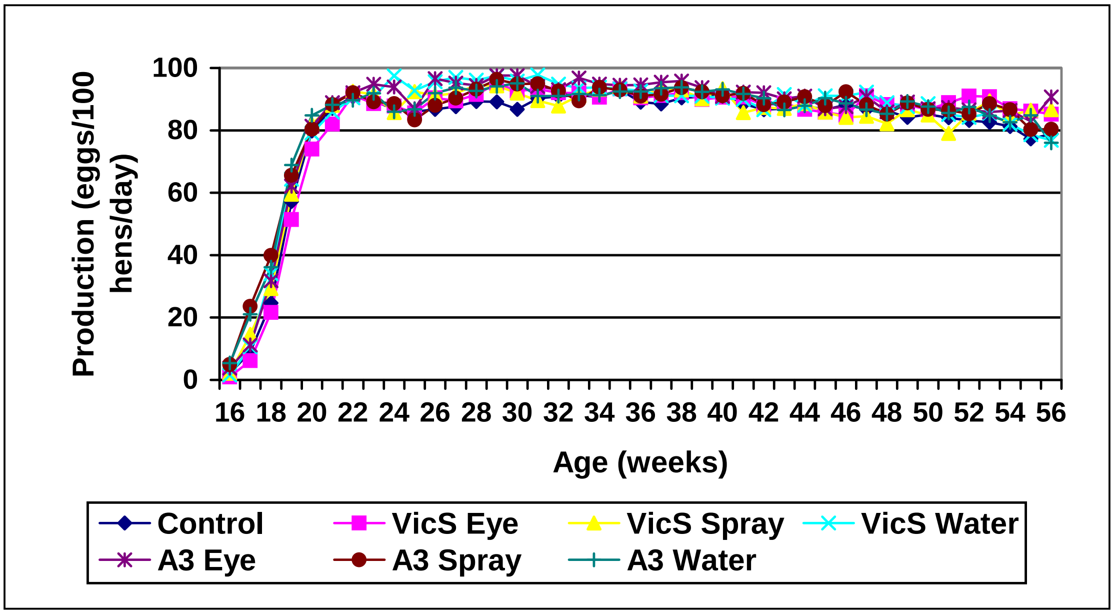
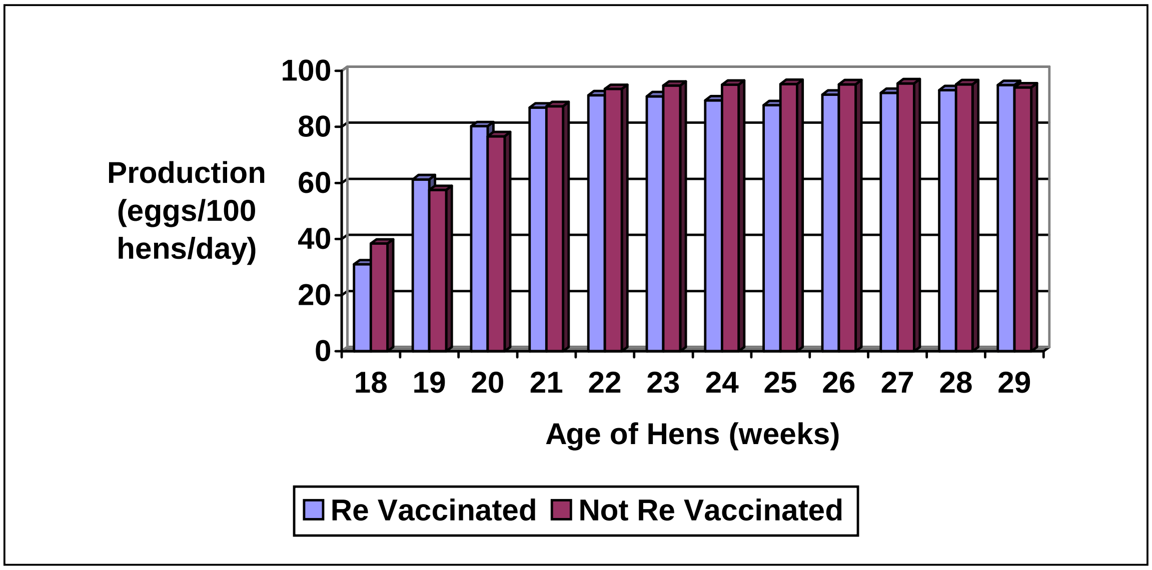


Figure 3.3 Production (eggs/100 hens/day) from 16 to 56 weeks of age in birds that were revaccinated regularly every 8 weeks (22, 30, 38, 46, 54 weeks) during lay or not revaccinated



3.4.3 Egg and Egg Shell Quality

The effects of initial vaccination treatments and regular revaccination for IB on egg and egg shell quality measurements: shell deformation (µm), shell breaking strength (Newtons), shell reflectivity (%), egg weight (g), albumen height (mm), Haugh units (HU), yolk colour (Roche Scale), shell weight (g), percentage shell (%) and shell thickness (µm); were determined at 20, 24, 28, 32, 36, 40, 44, 48, 52 and 56 weeks of age.

Egg weight varied significantly with age ($P<0.0001$), increasing to 44 weeks of age and then remaining relatively constant (Table 3.2). Egg weight was significantly affected by regular revaccination ($P<0.0001$), being slightly but significantly higher overall in the birds that were revaccinated regularly (Table 3.2). There was a significant effect of initial vaccination treatment ($P=0.0001$) with egg weight being lower for the initial control vaccination group (birds not vaccinated at all until 14 weeks of age) than for all other treatments (Table 3.3). In addition, there were significant interactions between age and regular revaccination ($P=0.0004$), and regular revaccination and initial vaccination treatment ($P<0.0001$). Full details of egg weight for each experimental group are contained in Table C6 in Appendix C.

Table 3.2 Egg weight at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	47.7 ± 0.5	46.8 ± 0.5	^{f}47.2 ± 0.3
24	54.6 ± 0.5	54.4 ±0.4	^{e}54.5 ± 0.3
28	59.6 ± 0.5	58.6 ± 0.4	^{d}59.1 ± 0.3
32	61.8 ± 0.4	57.9 ± 0.4	^{d}59.8 ± 0.3
36	62.8 ± 0.4	61.9 ± 0.3	^{c}62.3 ± 0.2
40	63.0 ± 0.4	61.9 ± 0.3	^{c}62.4 ± 0.3
44	64.0 ± 0.4	63.0 ± 0.4	^{a}63.5 ± 0.3
48	64.2 ± 0.4	63.6 ± 0.4	^{a}63.9 ± 0.3
52	62.8 ± 0.4	62.7 ± 0.4	^{b}62.7 ± 0.3
56	63.8 ± 0.5	63.4 ± 0.4	^{a}63.6 ± 0.3
All Ages	^{A}60.4 ± 0.2	^{B}59.4 ± 0.2	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.3 Egg weight at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments combined
Control	59.0 ± 0.5	58.6 ± 0.5	^{b}56.8 ± 0.3
VicS, eyedrop at day-old	60.3 ± 0.5	60.0 ± 0.5	^{a}60.1 ± 0.4
VicS, coarse spray at day-old	60.7 ± 0.5	59.4 ± 0.4	^{a}60.1 ± 0.4
VicS, in water at day-old	60.2 ± 0.5	59.7 ± 0.4	^{a}59.9 ± 0.3
A3, eyedrop at day-old	60.9 ± 0.5	59.6 ± 0.5	^{a}60.3 ± 0.4
A3, coarse spray at day-old	60.2 ± 0.5	60.3 ± 0.5	^{a}60.3 ± 0.3
A3, in water at day-old	61.8 ± 0.5	58.5 ± 0.4	^{a}60.2 ± 0.3
All Treatment Groups	^{A}60.4 ± 0.2	^{B}59.4 ± 0.2	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.4 Shell colour at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	32.8 ± 0.4	32.5 ± 0.5	^{d}32.6 ± 0.3
24	28.8 ± 0.3	28.9 ± 0.4	^{g}28.9 ± 0.2
28	30.4 ± 0.3	31.3 ± 0.4	^{f}30.9 ± 0.2
32	31.6 ± 0.3	31.9 ± 0.4	^{e}31.8 ± 0.2
36	33.4 ± 0.4	32.9 ± 0.3	^{d}33.2 ± 0.3
40	35.0 ± 0.4	35.6 ± 0.4	^{c}35.3 ± 0.3
44	35.4 ± 0.4	36.0 ± 0.4	^{bc}35.7 ± 0.3
48	37.0 ± 0.4	36.0 ± 0.4	^{a}36.5 ± 0.3
52	36.2 ± 0.4	36.3 ± 0.4	^{ab}36.2 ± 0.3
56	33.8 ± 0.4	33.0 ± 0.4	^{d}33.4 ± 0.3
All Ages	33.4 ± 0.1	33.4 ± 0.1	

Values are Mean ± S.E. Within a column values without the same superscript are significantly different from one another.

Table 3.5 Shell colour at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments combined
Control	34.2 ± 0.3	34.2 ± 0.4	^{a}34.2 ± 0.3
VicS, eyedrop at day-old	33.5 ± 0.3	33.9 ± 0.3	^{ab}33.7 ± 0.2
VicS, coarse spray at day-old	33.3 ± 0.4	35.1 ± 0.4	^{a}34.2 ± 0.3
VicS, in water at day-old	33.8 ± 0.3	32.7 ± 0.3	^{b}33.3 ± 0.2
A3, eyedrop at day-old	32.8 ± 0.4	32.1 ± 0.3	^{c}32.5 ± 0.3
A3, coarse spray at day-old	33.0 ± 0.3	33.1 ± 0.4	^{bc}33.1 ± 0.3
A3, in water at day-old	33.5 ± 0.4	32.9 ± 0.3	^{b}33.2 ± 0.3
All Treatment Groups	33.4 ± 0.1	33.4 ± 0.1	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Shell reflectivity (shell colour lightness) varied significantly with age ($P<0.0001$) (Table 3.4 and Table C7 in Appendix C). In general, shell colour increased to 24 weeks of age, after which it was generally lighter with age, except that, at 56 weeks, shell colour was similar to values recorded at 36 weeks of age. Shell colour was not significantly different between the birds that were revaccinated regularly during lay and those that were not. However, there was a significant difference among initial vaccination treatments ($P<0.0001$) (Table 3.5). Shell colour was lightest for the control, and VicS groups. The groups vaccinated initially with A3 had generally darker shells.

Shell breaking strength varied significantly with hen age ($P<0.0001$), with an increase between 20 and 24 weeks, following by a gradual decline (Table 3.6 and Table C8 in Appendix C). There was a significant ($P<0.0001$) effect of regular revaccination with breaking strength being slightly higher for the eggs produced by the birds that were not revaccinated regularly (Table 3.6). This “set-back” effect of revaccination can be seen in Figure 3.4. Initial vaccination treatment group had a significant effect (P=0.0007) with shell breaking strength being lowest for the group that received A3 strain vaccine by eyedrop and highest for the group receiving A3 in water (Table 3.7). There was a significant interaction between initial vaccination treatment and the presence or absence of regular revaccination during lay (P=0.0021). However, differences among vaccination groups were relatively small.

Figure 3.4 Shell breaking strength from 20 to 56 weeks of age in birds that were revaccinated every 8 weeks (22, 30, 38, 46, 54 weeks) or not revaccinated regularly during lay

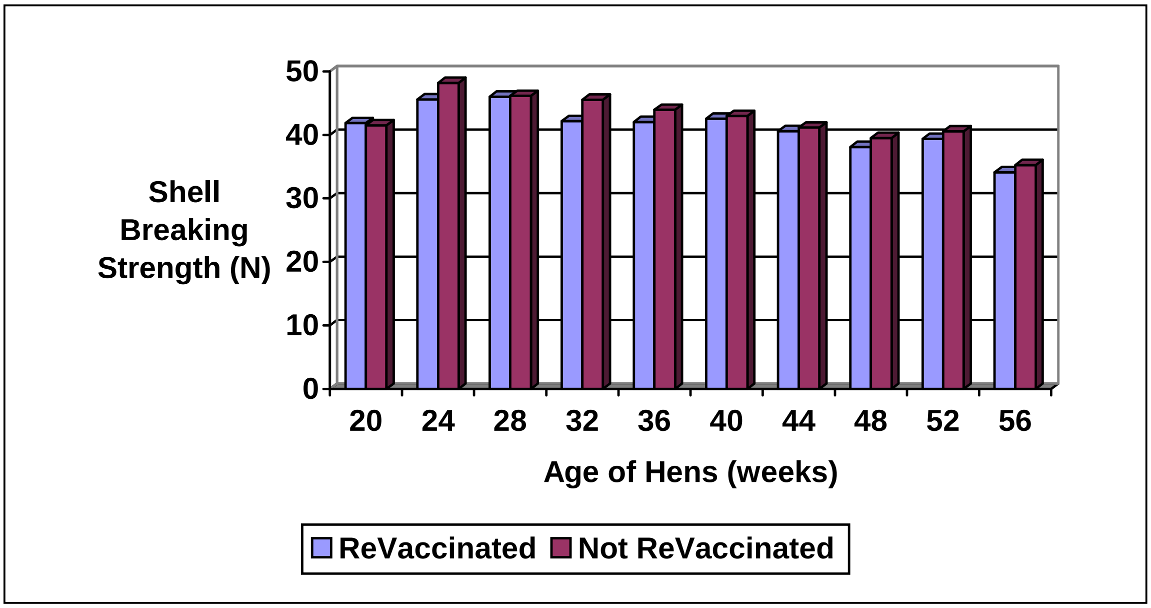


Table 3.6 Shell breaking strength at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	41.9 ± 0.8	41.5 ± 0.6	^{cd}41.7 ± 0.5
24	45.6 ± 0.6	48.2 ± 0.5	^{a}46.9 ± 0.4
28	46.0 ± 0.6	46.1 ± 0.5	^{a}46.1 ± 0.4
32	42.2 ± 0.6	45.5 ± 0.6	^{b}43.8 ± 0.5
36	42.0 ± 0.6	44.0 ± 0.7	^{bc}43.0 ± 0.5
40	42.6 ± 0.6	43.0 ± 0.6	^{bc}42.8 ± 0.4
44	40.6 ± 0.6	41.1 ± 0.6	^{de}40.9 ± 0.4
48	38.1 ± 0.7	39.5 ± 0.7	^{f}38.8 ± 0.5
52	39.4 ± 0.6	40.6 ± 0.6	^{ef}40.0 ± 0.4
56	34.2 ± 0.6	35.2 ± 0.6	^{g}34.7 ± 0.4
All Ages	^{B}41.2 ± 0.2	^{A}42.5 ± 0.2	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.7 Shell breaking strength at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments combined
Control	42.0 ± 0.6	42.3 ± 0.6	^{ab}42.2 ± 0.4
VicS, eyedrop at day-old	42.5 ± 0.6	41.8 ± 0.5	^{b}42.1 ± 0.4
VicS, coarse spray at day-old	41.4 ± 0.6	42.3 ± 0.6	^{bc}41.9 ± 0.4
VicS, in water at day-old	39.8 ± 0.5	42.9 ± 0.5	^{bc}41.4 ± 0.4
A3, eyedrop at day-old	39.5 ± 0.6	42.4 ± 0.5	^{c}40.9 ± 0.4
A3, coarse spray at day-old	40.8 ± 0.6	42.0 ± 0.6	^{bc}41.4 ± 0.4
A3, in water at day-old	42.7 ± 0.5	43.6 ± 0.6	^{a}43.2 ± 0.4
All Treatment Groups	^{B}41.2 ± 0.2	^{A}42.5 ± 0.2	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.8 Shell deformation at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	303.1 ± 5.8	280.6 ± 4.6	^{a}291.9 ± 3.8
24	274.9 ± 4.4	277.4 ± 4.9	^{b}276.2 ± 3.3
28	285.5 ± 6.3	276.8 ± 5.8	^{ab}281.1 ± 4.3
32	262.4 ± 7.0	263.6 ± 5.2	^{c}263.0 ± 4.4
36	281.7 ± 7.3	263.6 ± 5.7	^{b}272.7 ± 4.7
40	281.1 ± 8.6	271.2 ± 7.0	^{b}276.1 ± 5.5
44	258.8 ± 5.8	254.4 ± 6.5	^{c}256.6 ± 4.3
48	254.0 ± 6.9	261.3 ± 7.4	^{c}257.7 ± 5.0
52	260.2 ± 6.9	280.4 ± 8.2	^{bc}270.3 ± 5.4
56	244.7 ± 7.1	233.3 ± 6.7	^{d}239.0 ± 4.9
All Ages	270.5 ± 2.2	266.2 ± 2.0	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

There was a significant effect ($P<0.0001$) of age of hen on shell deformation and a significant interaction between age of hen and regular revaccination (Table 3.8 and Table C9 in Appendix C). However, there were no effects of regular revaccination or initial vaccination group on deformation. In general, shell deformation declined with hen age.

Shell weight varied significantly ($P<0.0001$) with hen age (Table 3.9 and Table C10 in Appendix C). Shell weight increased between 20 and 36 weeks of age after which there was a gradual decrease. There was no significant effect of regular revaccination during lay on shell weight. However, there were differences resulting from initial vaccination treatment ($P=0.0027$) (Table 3.10). In addition, there was a significant interaction between hen age and regular revaccination ($P=0.0034$). Shell weight was highest in the group that received A3 vaccine virus in water at day-old and lowest in the control.

Percentage shell varied significantly ($P<0.0001$) with the age of the birds (Table 3.11 and Table C11 in Appendix C). Percentage shell increased between 20 and 24 weeks of age after which it decreased. There was a significant effect of regular revaccination on percentage shell ($P<0.0001$) and a significant interaction between regular revaccination and initial vaccination treatment group ($P=0.0002$). This "set-back" effect of revaccination can be seen in Figure 3.5. However, there was no significant effect ($P=0.0538$) of initial vaccination treatment (Table 3.12).

Shell thickness varied significantly ($P<0.0001$) with hen age, increasing from 20 to 24 weeks and then declining slowly (Table 3.13 and Table C12 in Appendix C). There was a significant effect ($P<0.0001$) of regular revaccination on shell thickness with shell thickness being 5 microns greater for the birds that were not revaccinated regularly. Initial vaccination treatment also affected shell thickness ($P=0.0175$) (Table 3.14). In addition, there was a significant interaction between initial vaccination treatment and regular revaccination ($P=0.0004$). Shell thickness was higher in the group that received A3 in water initially than for all other groups.

Internal quality also varied in relation to hen age and vaccination treatment. Both albumen height and Haugh Units varied significantly ($P<0.0001$) with hen age (Tables 3.15 and 3.17 and Tables C13 and C14 in Appendix C). In general, albumen quality declined with age. However, the lowest albumen quality occurred at 32-44 weeks and this was most likely seasonally related. There were significant differences ($P<0.0001$) among initial vaccination treatment groups for both albumen height and Haugh Units (Tables 3.16 and 3.18), with albumen quality being highest for the control and lowest for the A3 water group. Regular revaccination during lay had no effect on albumen quality.

Table 3.9 Shell weight at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	4.74 ± 0.05	4.68 ± 0.05	[g]4.71 ± 0.04
24	5.73 ± 0.06	5.89 ± 0.05	[f]5.81 ± 0.04
28	6.23 ± 0.05	6.04 ± 0.04	[cd]6.19 ± 0.03
32	6.22 ± 0.04	6.04 ± 0.05	[d]6.13 ± 0.03
36	6.33 ± 0.04	6.45 ± 0.05	[a]6.39 ± 0.03
40	6.29 ± 0.05	6.29 ± 0.04	[b]6.29 ± 0.03
44	6.28 ± 0.04	6.24 ± 0.04	[bc]6.26 ± 0.03
48	6.20 ± 0.05	6.26 ± 0.04	[bc]6.23 ± 0.03
52	6.09 ± .05	6.26 ± 0.05	[cd]6.18 ± 0.04
56	5.99 ± 0.06	6.05 ± 0.07	[e]6.02 ± 0.04
All Ages	6.01 ± 0.02	6.03 ± 0.02	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.10 Shell weight at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments during lay
Control	5.89 ± 0.06	5.96 ± 0.05	[c]5.92 ± 0.04
VicS, eyedrop at day-old	6.05 ± 0.05	6.03 ± 0.06	[b]6.04 ± 0.04
VicS, coarse spray at day-old	6.03 ± 0.05	6.02 ± 0.05	[b]6.03 ± 0.04
VicS, in water at day-old	5.94 ± .0.05	6.09 ± 0.05	[b]6.02 ± 0.04
A3, eyedrop at day-old	5.99 ± 0.07	6.07 ± 0.05	[b]6.03 ± 0.04
A3, coarse spray at day-old	6.03 ± 0.05	6.01 ± 0.06	[b]6.02 ± 0.04
A3, in water at day-old	6.16 ± 0.05	6.07 ± 0.05	[a]6.11 ± 0.04
All Treatment Groups	6.01 ± 0.02	6.03 ± 0.02	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.11 Percentage shell at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	9.95 ± 0.09	10.03 ± 0.09	^{f}9.99 ± 0.07
24	10.50 ± 0.9	10.87 ± 0.07	^{a}10.69 ± 0.06
28	10.48 ± 0.06	10.50 ± 0.06	^{b}10.49 ± 0.04
32	10.09 ± 0.06	10.45 ± 0.07	^{c}10.27 ± 0.05
36	10.12 ± 0.07	10.42 ± 0.06	^{c}10.27 ± 0.05
40	9.97 ± 0.05	10.18 ± 0.06	^{d}10.07 ± 0.05
44	9.83 ± 0.06	9.93 ± 0.07	^{ef}9.88 ± 0.05
48	9.67 ± 0.07	9.87 ± 0.07	^{e}9.77 ± 0.05
52	9.72 ± 0.07	10.00 ± 0.08	^{ef}9.86 ± 0.05
56	9.43 ± 0.08	9.55 ± 0.09	^{g}9.49 ± 0.06
All Ages	^{B}9.98 ± 0.02	^{A}10.18 ± 0.03	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.12 Percentage shell at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments during lay
Control	10.01 ± 0.07	10.19 ± 0.07	10.10 ± 0.05
VicS, eyedrop at day-old	10.07 ± 0.06	10.08 ± 0.07	10.07 ± 0.05
VicS, coarse spray at day-old	9.98 ± 0.07	10.16 ± 0.06	10.07 ± 0.05
VicS, in water at day-old	9.89 ± 0.06	10.23 ± 0.06	10.06 ± 0.04
A3, eyedrop at day-old	9.85 ± 0.07	10.22 ± 0.06	10.03 ± 0.05
A3, coarse spray at day-old	10.04 ± 0.06	9.99 ± 0.07	10.02 ± 0.05
A3, in water at day-old	10.00 ± 0.06	10.41± 0.06	10.20 ± 0.05
All Treatment Groups	^{B}9.98 ± 0.02	^{A}10.18 ± 0.03	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Figure 3.5 Percentage shell from 20 to 56 weeks of age in birds that were revaccinated every 8 weeks (22, 30, 38, 46, 54 weeks) or not revaccinated regularly during lay

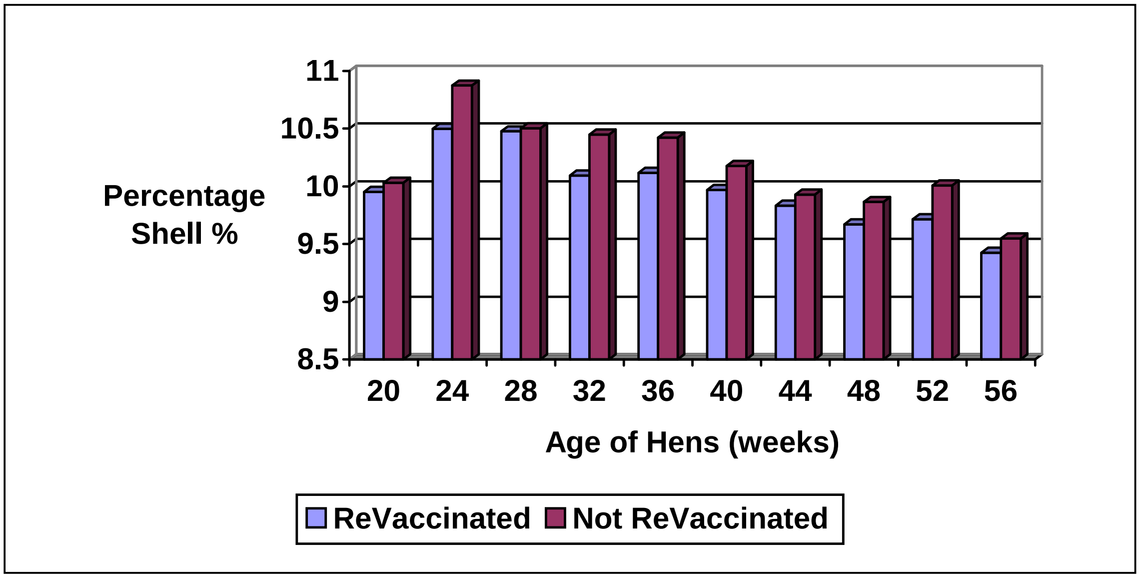


Table 3.13 Shell thickness at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	402.5 ± 3.0	402.8 ± 3.0	^{d}402.7 ± 2.1
24	437.7 ± 3.0	446.2 ± 2.1	^{a}442.0 ± 1.9
28	444.4 ± 2.4	441.6 ± 2.1	^{a}443.0 ± 1.6
32	433.6 ± 2.3	439.9 ± 3.4	^{b}436.7 ± 2.1
36	428.5 ± 2.2	441.6 ± 2.3	^{b}435.1 ± 1.6
40	435.1 ± 2.2	439.0 ± 2.0	^{b}437.0 ± 1.5
44	435.6 ± 2.2	436.7 ± 2.1	^{b}436.2 ± 1.5
48	430.3 ± 2.5	435.6 ± 2.4	^{b}433.0 ± 1.7
52	430.8 ± 2.8	441.3 ± 2.8	^{b}436.1 ± 2.0
56	425.4 ± 2.9	427.7 ± 3.5	^{c}426.5 ± 2.3
All Ages	^{B}430.4 ± 0.9	^{A}435.3 ± 0.9	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.14 Shell thickness at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments during lay
Control	430.6 ± 2.6	434.9 ± 2.2	^{b}432.8 ± 1.7
VicS, eyedrop at day-old	431.9 ± 2.1	430.5 ± 2.7	^{b}431.2 ± 1.7
VicS, coarse spray at day-old	430.4 ± 2.4	431.3 ± 2.4	^{b}430.8 ± 1.6
VicS, in water at day-old	428.2 ± 2.1	437.6 ± 2.0	^{b}432.9 ± 1.5
A3, eyedrop at day-old	423.3 ± 2.4	439.4 ± 1.9	^{b}431.3 ± 1.6
A3, coarse spray at day-old	433.4 ± 2.3	432.8 ± 2.7	^{b}433.1 ± 1.8
A3, in water at day-old	435.4 ± 2.1	441.1 ± 2.2	^{a}438.2 ± 1.5
All Treatment Groups	^{B}430.4 ± 0.9	^{A}435.3 ± 0.9	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.15 Albumen height at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	8.02 ± 0.11	7.97 ± 0.10	^{a}8.00 ± 0.08
24	8.16 ± 0.10	8.11 ± 0.08	^{a}8.14 ± 0.07
28	7.25 ± 0.09	7.35 ± 0.08	^{cd}7.30 ± 0.06
32	7.19 ± 0.09	7.23 ± 0.09	^{d}7.21 ± 0.07
36	6.93 ± 0.10	7.05 ± 0.09	^{e}6.99 ± 0.07
40	6.45 ± 0.11	6.44 ± 0.09	^{f}6.44 ± 0.07
44	6.83 ± 0.10	6.94 ± 0.11	^{e}6.89 ± 0.07
48	7.36 ± 0.10	7.53 ± 0.11	^{c}7.45 ± 0.07
52	7.68 ± 0.12	7.68 ± 0.12	^{b}7.68 ± 0.09
56	7.52 ± 0.12	7.52 ± 0.12	^{bc}7.52 ± 0.08
All Ages	7.34 ± 0.04	7.38 ± 0.03	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.16 Albumen height at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments combined
Control	7.64 ± 0.10	7.66 ± 0.08	[a]7.65 ± 0.07
VicS, eyedrop at day-old	7.60 ± 0.10	7.45 ± 0.09	[ab]7.52 ± 0.07
VicS, coarse spray at day-old	7.58 ± 0.09	7.35 ± 0.09	[b]7.47 ± 0.06
VicS, in water at day-old	7.20 ± 0.10	7.38 ± 0.09	[cd]7.29 ± 0.07
A3, eyedrop at day-old	7.21 ± 0.09	7.60± 0.09	[bc]7.41 ± 0.06
A3, coarse spray at day-old	7.10 ± 0.08	7.35 ± 0.09	[d]7.23 ± 0.06
A3, in water at day-old	7.03 ± 0.09	6.85 ± 0.09	[e]6.94 ± 0.06
All Treatment Groups	7.34 ± 0.04	7.38 ± 0.03	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.17 Haugh units at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated regularly	Not revaccinated	All birds
20	92.2 ± 0.7	92.4 ± 0.6	[a]92.3 ± 0.4
24	91.2 ± 0.6	91.1 ± 0.5	[a]91.1 ± 0.4
28	84.9 ± 0.6	85.8 ± 0.5	[bc]85.4 ± 0.4
32	83.7 ± 0.6	84.9 ± 0.6	[c]84.3 ± 0.4
36	84.5 ± 0.6	82.7 ± 0.6	[d]82.1 ± 0.4
40	77.8 ± 0.8	78.3 ± 0.7	[f]78.1 ± 0.5
44	80.2 ± 0.8	81.3 ± 0.7	[e]80.8 ± 0.5
48	83.6 ± 0.8	85.0 ± 0.7	[c]84.3 ± 0.5
52	85.8 ± 0.8	85.9 ± 0.8	[b]85.9 ± 0.6
56	84.5 ± 0.9	84.8 ± 0.8	[bc]84.6 ± 0.6
All Ages	84.5 ± 0.3	85.2 ± 0.2	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.18 Haugh units at 20-56 weeks of age for birds receiving different initial vaccination protocols and either revaccinated regularly or not revaccinated after 14 weeks.

Group	Revaccinated regularly	Not revaccinated	Both treatments combined
Control	84.5 ± 0.3	87.3 ± 0.5	[a]87.0 ± 0.4
VicS, eyedrop at day-old	85.2 ± 0.7	85.5 ± 0.6	[b]85.8 ± 0.5
VicS, coarse spray at day-old	86.1 ± 0.7	84.9 ± 0.6	[b]85.5 ± 0.5
VicS, in water at day-old	83.6 ± 0.7	85.2 ± 0.6	[c]84.4 ± 0.5
A3, eyedrop at day-old	83.6 ± 0.7	86.5 ± 0.6	[bc]85.0 ± 0.4
A3, coarse spray at day-old	83.3 ± 0.6	84.8 ± 0.6	[c]84.1 ± 0.4
A3, in water at day-old	82.4 ± 0.6	82.1 ± 0.6	[d]82.3 ± 0.4
All Treatment Groups	84.5 ± 0.3	85.2 ± 0.2	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Table 3.19 Yolk colour score at 20-56 weeks of age for birds revaccinated regularly and those not revaccinated after 14 weeks.

Age (weeks)	Revaccinated Regularly	Not Revaccinated	All Birds
20	9.76 ± 0.07	9.58 ± .0.07	[g]9.67 ± 0.05
24	11.10 ± 0.08	11.31 ± 0.08	[e]11.20 ± 0.05
28	11.98 ± 0.06	11.42 ± 0.06	[c]11.70 ± 0.05
32	11.27 ± 0.07	11.19 ± 0.08	[e]11.23 ± 0.05
36	11.76 ± 0.07	11.91 ± 0.06	[b]11.83 ± 0.05
40	11.64 ± 0.07	11.71 ± 0.06	[c]11.68 ± 0.05
44	11.95 ± 0.06	12.01 ± 0.06	[a]11.98 ± 0.04
48	11.33 ± 0.06	11.55 ± 0.06	[d]11.44 ± 0.04
52	10.88 ± 0.08	10.51 ± 0.07	[f]10.70 ± 0.05
56	10.65 ± 0.07	10.98 ± 0.06	[f]10.82 ± 0.05
All Ages	11.24 ± 0.03	11.22 ± 0.03	

Values are Mean ± S.E. Within a column or across a row, values without the same superscript are significantly different from one another.

Yolk colour varied significantly ($P<0.0001$) with hen age but was not significantly affected by initial vaccination treatment group or regular revaccination during lay (Table 3.19 and Table C15 in Appendix C). There was no clear pattern of variation in yolk colour which may indicate that the variation with hen age was related to differences in feed intake.

3.4.4 Faecal Moisture

Faecal moisture was measured in samples collected over a 24 hour period, one and two weeks following re-vaccination. At 16 weeks, the only effect was that the control group, which had not been vaccinated prior to 14 weeks of age, tended (p=.0544) to have wetter faeces at 16 weeks of age, two weeks following vaccination for the first time (Table 3.20).

Table 3.20 Faecal moisture of control and vaccinated birds at 16 weeks of age

Treatment Group	Control	VicS eye	VicS spray	VicS Water	A3 eye	A3 spray	A3 water
Faecal Moisture %	74.7 ± 2.3	67.4 ± 2.2	68.2 ± 0.9	71.5 ± 1.1	70.6 ± 2.3	67.8 ± 2.1	71.1 ± 1.7

Mean ± S.E.

For the excreta collections conducted at 23-24, 31-32, 39-40, 47-48 and 55-56 weeks, one and two weeks following regular revaccination of half the birds, there were no overall significant effects of regular revaccination or initial vaccination group (see Table C16 in Appendix C). However, there was a significant difference between one and two weeks post vaccination ($P<0.0001$), as shown in Table 3.21. There was a significant difference among the different ages of hens at the time of excreta collection (see Table C16 in Appendix C).

Table 3.21 Faecal moisture (%) of revaccinated and not revaccinated groups at 1 and 2 weeks postvaccination

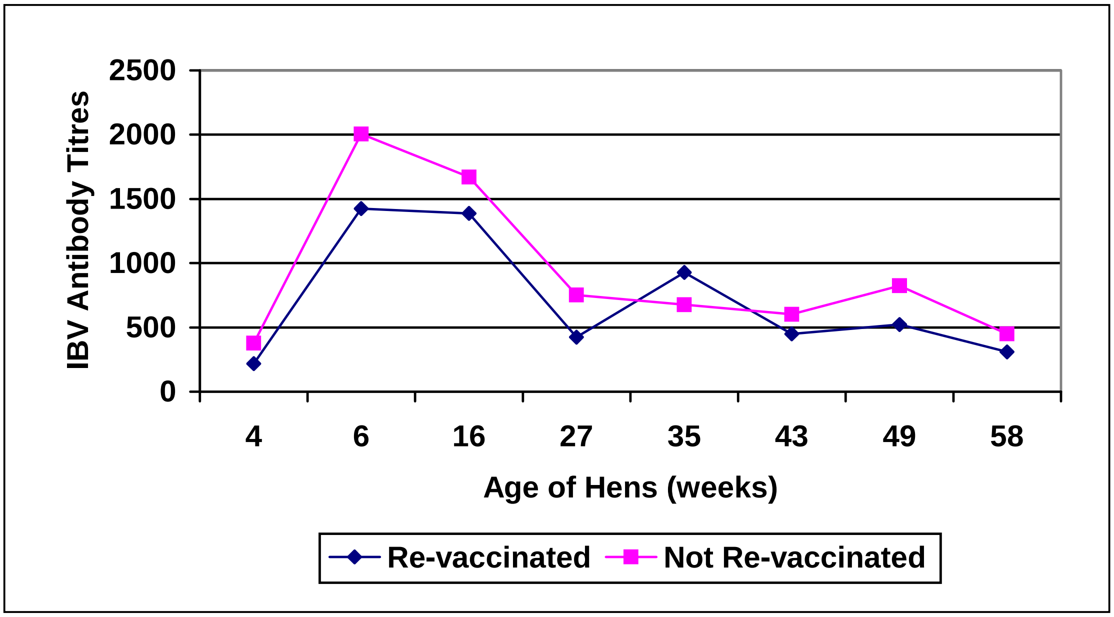
	Weeks following revaccination	
Vaccination Treatment	Week 1	Week 2
Revaccinated every 8 weeks	72.6 ± 0.3	70.6 ± 0.4
Not revaccinated regularly	71.9 ± 0.4	72.4 ± 0.4
Both groups combined	[a]72.3 ± 0.2	[b]71.5 ± 0.3

Mean ± S.E. Values in a row without the same superscripts are significantly different from one another.

3.4.5 IB Titre Levels, Blood and Plasma Measurements

Titre levels of non-revaccinated and revaccinated birds could be seen on Figures 3.6, 3.7 and 3.8 (see also Table C17 in Appendix C). The titres for bloods taken at 4 and 6 weeks are repeated here to illustrate the changes in titres in early lay. There was no significant effect of vaccination treatment on titre levels. However, titre levels varied with the age of bird, being greatest at 6-16 weeks of age.

Figure 3.6 IBV antibody titres of birds that were revaccinated or not revaccinated regularly during lay



There were no significant effects of initial vaccination treatment on haematocrit, or the plasma concentrations of sodium, potassium and ionised calcium (see Tables C17-C20 in Appendix C). In addition, there were no significant effects of regular revaccination on any of the blood parameters. However, there were significant differences among the ages of hen ($P<0.0001$) for haematocrit, plasma sodium, potassium and ionised calcium, as shown in Table 3.22.

Figure 3.7 IBV antibody titres of birds that were revaccinated regularly every 8 weeks during lay

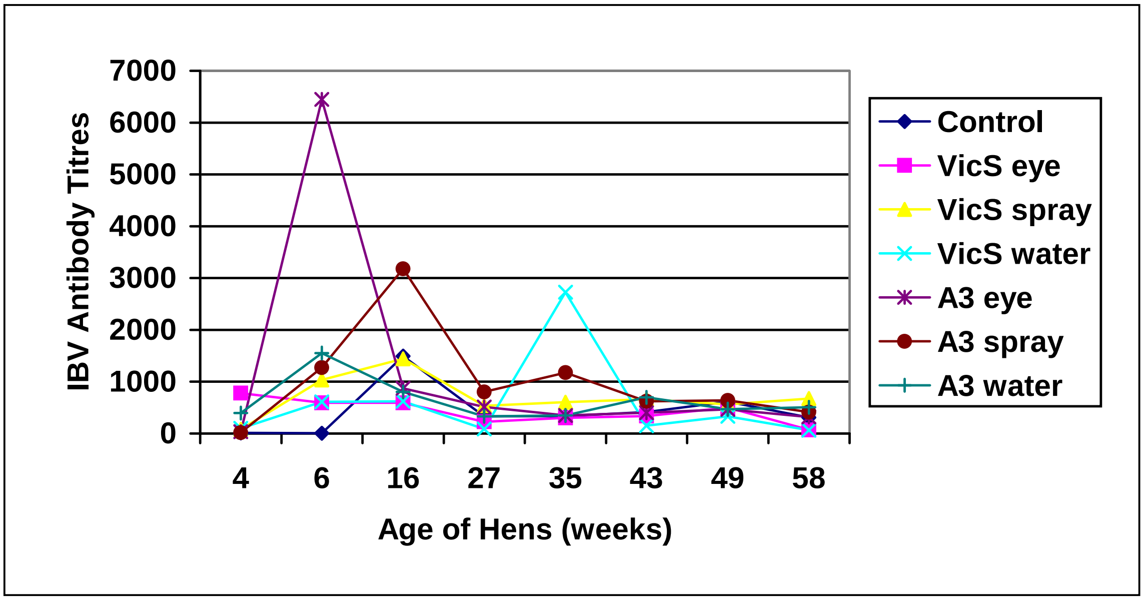


Figure 3.8 IBV antibody titres of birds that were not revaccinated regularly during lay

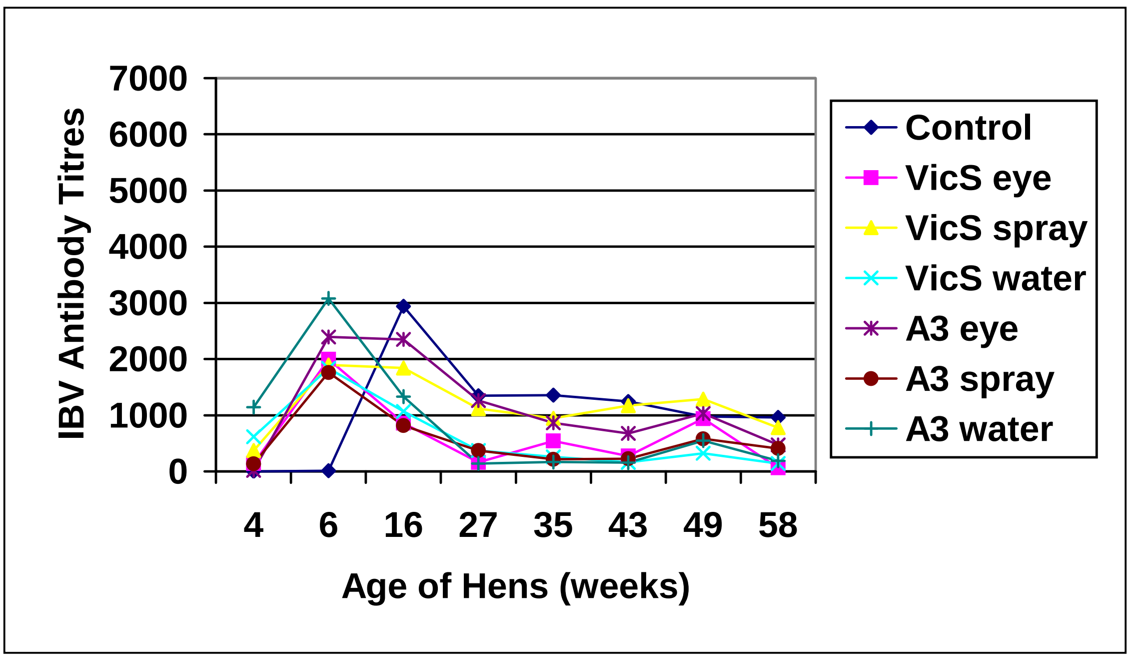


Table 3.22 Haematocrit and plasma concentrations of sodium, potassium and ionised calcium for hens at different ages during lay

Age of Hens	Wk 16	Wk 27	Wk 35	Wk 43	Wk 49	Wk 58
Measurement						
Haematocrit %	^{c}29.2 ± 0.8	^{bc}30.1 ± 0.3	^{a}31.5 ± 0.3	^{a}30.6 ± 0.4	^{a}31.9 ± 0.2	^{c}29.1 ± 0.2
Plasma Na mM	^{c}146.6 ± 0.3	^{c}147.5 ± 0.3	^{a}152.2 ± 0.3	^{b}149.4 ± 0.4	^{a}152.7 ± 0.3	^{d}135.5 ± 0.6
Plasma K mM	^{a}5.84 ± 0.05	^{c}5.00 ± 0.05	^{b}5.38 ± 0.07	^{b}5.31 ± 0.06	^{c}5.01 ± 0.05	^{b}5.39 ± 0.05
Plasma Ca^{++} mM	^{d}1.49 ± 0.02	^{c}1.57 ± 0.01	^{a}1.73 ± 0.01	^{b}1.63 ± 0.01	^{a}1.73 ± 0.01	^{e}1.26 ± 0.01

Means ± S.E. Values in rows with different superscripts differ significantly

3.5 Discussion and Conclusions

All the variables measured, production , egg and egg shell quality, excreta moisture, IBV antibody titres, haematocrit and plasma electrolyte concentrations varied significantly with hen age. Initial vaccination treatment had no significant effects on the egg quality measurements of deformation, percentage shell and yolk colour. Some measures of egg shell quality, egg weight, shell colour and shell weight were less favourable in the control birds that were not vaccinated for IBV until 14 weeks of age. In contrast, some measures (shell breaking strength, shell weight, shell thickness) were highest for the group that initially received A3 vaccine in water.

However, regular revaccination had some deleterious effects on production and egg shell quality. Production was lower overall for the birds that were revaccinated regularly during lay. In addition, shell breaking strength, percentage shell and shell thickness were all higher in the birds that were not revaccinated regularly. However, egg weight was higher in the birds that were revaccinated regularly.

The group of birds which had been vaccinated at day-old with A3 strain IB vaccine in water tended to have lower albumen height and Haugh Units. Results suggest that there is little advantage in regularly revaccinating laying hens for IB virus, provided that they have received appropriate vaccination during the rearing phase. However, more information is required about the correlation between blood IB titre levels and protection against intercurrent IB infection before recommendations can be made to the Australian industry.

4. Trial 1: effects of vaccine strain and route of vaccine administration in providing protection against infectious bronchitis virus (IBV) in laying hens – Moult and Exposure to T-Strain IBV

4.1 Introduction

Moulting of birds is conducted under a variety of circumstances such as when there is a strong demand for eggs or when the cost of replacing a flock is high in relation to returns. However, moulting appears to be less common with the imported strains of birds than it was with the former Australian strains although this may be due to the changed market forces resulting from deregulation of the egg industry. In response to producer enquiries concerning IBV vaccination and moult, this study was conducted to assess the relative benefits of revaccinating birds prior to moult or following moult.

4.2 Objectives

There were two main objectives of this study:

1. The first objective was to answer the question: which gives the best result in terms of production and egg and egg shell quality – revaccination for IBV prior to an induced moult or revaccination for IBV following an induced moult. Other measurements conducted were manure moisture following revaccination, blood and plasma parameters.
2. The second objective was to challenge the flock with the standard challenge virus, T-strain IBV and to determine the level of protection afforded by the previous vaccination treatments

4.3 Methodology

At 57 weeks of age, birds were moved to individual cages for revaccination either before or after an induced moult. Half of the birds were moulted at 57 weeks by removal of artificial light and feeding whole grain barley and shell grit for a period of 5 weeks. At 62 weeks of age, all birds were revaccinated by coarse spray with VicS IBV. Birds that had been moulted were then placed on a normal commercial layer diet and the other half of the birds were placed on whole grain barley and shell grit for a period of 5 weeks (until 67 weeks).

At 77 wks of age, birds were exposed to T strain IBV. The challenge virus was purchased from Dr. Jagoda Ignjatovic of the CSIRO Australian Animal Health Laboratory in Geelong. The virus was then passaged once in fertile eggs and titrated in eggs. The titre, determined after the method of Reed and Muench (1938), was 10^7 EID_{50}/mL. The challenge virus was administered by eyedrop to one bird in ten. As the birds were housed in back-to-back cages, birds were alternated so that there were never any more that 4 birds in between eyedropped birds. It was estimated that each bird received a dose of approximately 10^4 CD_{50} T-strain IBV. Virus from the same stock had been shown previously to cause mortality and kidney damage in unvaccinated cockerels (Chapter 1 and Sulaiman *et al.,* 2001a,b).

There were now 28 groups in total: 7 initial vaccination treatments, birds revaccinated regularly during lay and those which were not, as well as late revaccination of all birds either before or after an induced moult. For each group, egg production, egg weight and the external appearance of the eggs were recorded daily. Faecal moisture was measured 1 and 2 weeks post revaccination. Every 4 weeks, 21 eggs of each group from each shed were collected for egg and egg shell quality measurements (egg weight, shell reflectivity, shell breaking strength, deformation, shell weight, shell thickness, percentage shell, albumen height, Haugh Units, yolk colour score). Blood samples were taken, from 5 birds from each group, 3 weeks after revaccination (65 weeks of age), just prior to challenge (77 weeks of age), and two and three weeks following challenge (79 and 80 weeks of age) for determination of haematocrit, plasma electrolytes and antibody titres.

Analysis of Variance was used to test the effect of vaccination treatment, regular revaccination during production and the timing of moult on each measured parameter. Fisher's protected LSD was utilized to separate means when significant effects were observed. Statements of statistical significance were based on P<0.05.

4.4 Detailed Results

4.4.1 Effect of Moult

4.4.1.1 Hen-day Production
Overall, there were significant main effects on hen-day production of initial vaccination treatment and regular revaccination from 57 to 73 weeks of age (see Tables D1 and D2 in Appendix D). The birds that had been revaccinated regularly for IBV during lay had slightly lower production at 57-73 weeks (57.9 eggs/hen/100 days) than the birds that had not been revaccinated (59.2 eggs/hen/100 days). Control (no vaccination until 14 weeks) and the VicS eye group had lower production than the other groups (Table 4.1). There was a significant interaction between initial vaccination treatment and the timing of moult with production being more variable for the birds that had not been revaccinated regularly. There was also a significant interaction between whether or not birds had been revaccinated regularly during lay and the timing of the moult. For birds that had been revaccinated regularly during lay, production was higher for those moulted after revaccination at 62 weeks, whereas for birds not revaccinated regularly, production was higher when moult preceded revaccination. However, there was no significant main effect of timing of moult on overall hen-day production.

Table 4.1 Effect of initial vaccination treatment on hen-day production (eggs/hen/100 days) before, during and after an induced moult.

Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water
^{d}55.8 ± 1.85	^{d}55.8 ± 1.79	^{ab}60.3 ± 1.83	^{c}58.2 ± 1.77	^{ab}60.3 ± 1.89	^{bc}58.6 ± 1.91	^{a}60.6 ± 1.91

Mean ± S.E. Values in a row with different superscripts differ significantly

4.4.1.2 Egg and Egg Shell Quality
Detailed results for egg and egg shell quality are contained in Tables D3-D12 in Appendix D. Only one of the moult treatment groups was sampled for the egg collections at 62 weeks (birds moulted after revaccination), 64 and 68 weeks (birds moulted prior to revaccination). At these times, there were very few statistically significant effects on egg and egg shell quality. However, at 72 and 78 weeks of age, eggs were collected from all birds. A general finding was that egg and egg shell quality were better in the birds that were moulted after revaccination, than in birds that were moulted prior to revaccination (Table 4.2). The improved breaking strength and Haugh Units in birds moulted after revaccination were seen also at 72 weeks of age. If the two groups were compared at 10 weeks following moult (72 weeks of age for the birds moulted prior to revaccination, 78 weeks of age in the birds moulted following revaccination), the latter group had significantly better shell breaking strength, shell weight, percentage shell and shell thickness. There were also some effects at 72 and 78 weeks of initial vaccination treatment with breaking strength being generally higher in the control birds and those vaccinated initially with A3 strain.

Table 4.2 Effect of timing of moult on egg and egg shell quality at 72 weeks (Mean±SE)

	Moult Before Revaccination	Moult After Revaccination
Egg Weight g	64.8 ± 0.3	64.6 ± 0.3
Shell Reflectivity %	32.2 ± 0.2	32.2 ± 0.3
Breaking Strength N	$^{b}34.3 \pm 0.6$	$^{a}37.0 \pm 0.6$
Deformation µm	269.1 ± 7.8	268.8 ± 7.0
Shell Weight g	6.18 ± 0.03	6.15 ± 0.03
Percentage Shell %	9.54 ± 0.05	9.54 ± 0.04
Shell Thickness	430.3 ± 1.8	427.4 ± 1.6
Albumen Height	7.90 ± 0.09	8.12 ± 0.10
Haugh Units	86.5 ± 0.6	84.5 ± 0.7
Yolk Colour	$^{a}10.99 \pm 0.05$	$^{b}10.70 \pm 0.06$

Values in a row with different superscripts differ significantly

Table 4.3 Effect of timing of moult on egg and egg shell quality at 78 weeks (Mean±SE)

	Moult Before Revaccination	Moult After Revaccination
Egg Weight g	65.5 ± 0.4	64.5 ± 0.4
Shell Reflectivity %	$^{a}35.6 \pm 0.6$	$^{b}31.9 \pm 0.4$
Breaking Strength N	37.0 ± 0.8	39.1 ± 0.9
Deformation µm	258.2 ± 8.7	279.3 ± 10.1
Shell Weight g	$^{b}6.18 \pm 0.05$	$^{a}6.33 \pm 0.05$
Percentage Shell %	$^{b}9.46 \pm 0.08$	$^{a}9.82 \pm 0.07$
Shell Thickness	$^{b}427.8 \pm 2.8$	$^{a}440.4 \pm 2.8$
Albumen Height	$^{b}7.39 \pm 0.14$	$^{a}7.80 \pm 0.13$
Haugh Units	$^{b}82.9 \pm 1.0$	$^{a}86.1 \pm 0.8$
Yolk Colour	$^{b}10.02 \pm 0.08$	$^{a}10.25 \pm 0.09$

Values in a row with different superscripts differ significantly

Table 4.4 Comparison of egg quality in birds moulted prior to revaccination at 72 weeks and those moulted following revaccination at 78 weeks (Mean±SE)

	Moult Before Revaccination At 72 weeks	Moult After Revaccination At 78 weeks
Egg Weight g	64.8 ± 0.3	64.5 ± 0.4
Shell Reflectivity %	32.2 ± 0.2	31.9 ± 0.4
Breaking Strength N	$^{b}34.3 \pm 0.6$	$^{a}39.1 \pm 0.9$
Deformation µm	269.1 ± 7.8	279.3 ± 10.1
Shell Weight g	$^{b}6.18 \pm 0.03$	$^{a}6.33 \pm 0.05$
Percentage Shell %	$^{b}9.54 \pm 0.05$	$^{a}9.82 \pm 0.07$
Shell Thickness	$^{b}430.3 \pm 1.8$	$^{a}440.4 \pm 2.8$
Albumen Height	7.90 ± 0.09	7.80 ± 0.13
Haugh Units	86.5 ± 0.6	86.1 ± 0.8
Yolk Colour	$^{a}10.99 \pm 0.05$	$^{b}10.25 \pm 0.09$

Values in a row with different superscripts differ significantly

4.4.1.3 Faecal Moisture

Faecal moisture was measured in samples collected over a 24 hour period, one and two weeks following revaccination. Excreta moisture was significantly higher (P=0.0015) for the birds that had previously been revaccinated regularly (78.3%) than for the birds which were not revaccinated (74.5%). Excreta moisture was significantly higher in the birds that were moulted after revaccination (83.8%) than those moulted before revaccination (69.8%) (P<0.0001). There was also a significant effect (P=0.0223) of initial vaccination treatment with excreta moisture being highest in the control and VicS spray groups (Table 4.3 and Table D13 in Appendix D)

Table 4.5 Initial vaccination treatment and excreta moisture following late revaccination

Initial Vaccination Treatment Group	Excreta Moisture Following Revaccination at 62 weeks
Control	$^{a}79.9 \pm 1.3$
VicS, eyedrop at day-old	$^{ab}77.4 \pm 2.0$
VicS, coarse spray at day-old	$^{a}79.1 \pm 1.9$
VicS, in water at day-old	$^{b}74.8 \pm 1.9$
A3, eyedrop at day-old	$^{b}75.0 \pm 1.6$
A3, coarse spray at day-old	$^{b}74.7 \pm 1.8$
A3, in water at day-old	$^{b}74.6 \pm 1.8$

Mean ± S.E. Values in a column without the same superscript are significantly different

4.4.1.4 Blood Electrolytes

Haematocrit and the plasma concentrations of sodium, potassium and calcium were significantly affected by the age of the birds, during the moult experiment (Table 4.4 and Tables D14-D17 in Appendix D). Haematocrit decreased from 58 to 77 weeks of age, at the same time as the plasma concentrations of sodium, potassium and ionized calcium increased.

Table 4.6 Haematocrit and plasma electrolytes and hen age.

	Age of Hens		
	58 weeks	65 weeks	77 weeks
Haematocrit %	$^{a}33.2 \pm 0.4$	$^{b}30.9 \pm 0.5$	$^{c}29.3 \pm 0.3$
Plasma Na mM	$^{c}145.4 \pm 0.4$	$^{b}148.9 \pm 0.4$	$^{a}154.4 \pm 0.4$
Plasma K mM	$^{c}4.61 \pm 0.06$	$^{b}5.09 \pm 0.06$	$^{a}5.54 \pm 0.05$
Plasma Ca^{++} mM	$^{b}1.44 \pm 0.02$	$^{a}1.78 \pm 0.03$	$^{a}1.79 \pm 0.01$

Mean ± S.E.
Revaccination occurred at 62 weeks
Values in a row with different superscripts differ significantly

There was also a significant effect of initial vaccination treatment on plasma sodium concentration from 58-77 weeks, with plasma sodium being highest in the VicS spray group, lowest in the VicS water and all the A3 groups, with the control and VicS eyedrop groups being intermediate.

4.4.1.5 IB Titre Levels

There was no significant effect of initial vaccination treatment, regular revaccination or timing of moult on titre levels. However, titres were significantly higher for all treatment groups at 65 weeks of age (558) than at 58 weeks (379), with 77 weeks being intermediate (473) (Table D18 in Appendix D).

4.4.2 Detailed Results: Effect of Challenge

4.4.2.1 Production

Production over the period of weeks 74-81 was significantly affected by initial vaccination treatment (P=0.0020), whether or not the birds were revaccinated regularly during lay (P<0.0001) and the timing of the moult in relation to revaccination (P<0.0001). Production was higher in the birds that were not revaccinated regularly during lay (76.4 eggs/100 hens/day) than for the birds that were revaccinated regularly every 8 weeks (71.7 eggs/100 hens/day). Production was higher at 74-81 weeks for the birds that were revaccinated prior to the induced moult (76.3 eggs/100 hens/day) than for the birds that were revaccinated after the induced moult (71.9 eggs/100 hens/day). Production was highest in the A3 water group and lowest in the VicS eyedrop and VicS spray groups (Table 4.5). There was also a significant effect of hen age, with production generally declining as the birds got older (Table 4.6).

Table 4.7 Production in relation to initial vaccination treatment group

Initial Vaccination Treatment Group	Production from 74 to 81 weeks of age
Control	$^{b}76.8 \pm 1.3$
VicS, eyedrop at day-old	$^{b}75.3 \pm 1.4$
VicS, coarse spray at day-old	$^{b}78.4 \pm 1.4$
VicS, in water at day-old	$^{b}72.1 \pm 1.5$
A3, eyedrop at day-old	$^{ab}74.9 \pm 1.4$
A3, coarse spray at day-old	$^{b}72.5 \pm 1.6$
A3, in water at day-old	$^{a}71.7 \pm 2.0$

Mean ± S.E. Values in a column without the same superscript are significantly different

Table 4.8 Production at 74-81 weeks of age

Hen Age (weeks)	Production (eggs/100 hens/day)
74	$^{ab}76.8 \pm 1.3$
75	$^{abc}72.3 \pm 1.4$
76	$^{a}78.4 \pm 1.4$
77	$^{cd}72.1 \pm 1.5$
78	$^{abcd}74.9 \pm 1.4$
79	$^{bcd}72.5 \pm 1.4$
80	$^{c}71.7 \pm 2.0$
81	$^{d}70.8 \pm 2.0$

Mean ± S.E. Values in a column without the same superscript are significantly different

4.4.2.2 Egg Abnormalities

The incidence of external egg abnormalities was monitored from 74 to 81 weeks of age so that any effect of the challenge with T-strain IBV at 77 weeks could be identified. The total number of abnormal eggs was significantly affected by the age of the birds and increased markedly (P<0.0001) following challenge at 77 weeks (Table 4.7). In addition, there were significant differences among initial vaccination treatment groups (P=0.0004) and differences in relation to the timing of the induced moult (P=0.0001). The incidence of abnormal eggs was significantly higher in the birds that were moulted before revaccination (19.2%), as compared with the birds that were moulted after revaccination (15.7%).

Table 4.9 The incidence of abnormal eggs at 74-81 weeks of age

	Type of Egg Abnormality			
Age of Hens (weeks)	Abnormal % Total Eggs Laid	Broken Eggs % abnormal eggs	Soft-Shelled Eggs % abnormal eggs	White-banded % abnormal eggs
74	7.6	23.3	0.08	0.47
75	5.8	20.3	0.94	2.37
76	5.1	23.0	0.90	1.79
77	9.2	23.6	0.37	2.92
78	22.2	23.4	0.42	1.89
79	22.6	28.7	0.47	3.55
80	29.0	26.5	0.68	2.70
81	38.4	25.2	0.10	2.33

4.4.2.3 Body Weight and Kidney Weights

There were no differences among any of the treatment groups for body weight of birds sacrificed for collection of kidney tissue (see Table D20 in Appendix D). There were no significant differences in the weights of left and right kidneys from each bird (Tables D23-D26). Table 4.8 summarises the body weights and kidney weights as a percentage of body weight for the kidneys removed prior to challenge and then at weekly intervals for 5 weeks following challenge. Total kidney weight as a percentage of body weight was not significantly affected by initial vaccination treatment, regular revaccination during lay, timing of moult or time in relation to challenge with T-strain infectious bronchitis virus.

Table 4.10 Summary of body weight (BW), percentages of total kidney, right and left kidney of BW at weeks post exposure to T strain IBV.

Week	Body weight (BW)(g)	Total kidney (%BW)	Right kidney (%BW)	Left kidney (%BW)
Pre-Challenge	2109.89^{a}	0.70^{a}	0.34^{a}	0.34^{a}
1	2052.85^{a}	0.64^{bc}	0.32^{b}	0.33^{a}
2	2116.28^{a}	0.62^{c}	0.31^{b}	0.31^{a}
3	2114.10^{a}	0.65^{bc}	0.32^{b}	0.33^{a}
4	2156.48^{a}	0.63^{bc}	0.31^{b}	0.32^{a}
5	2169.86^{a}	0.66^{ab}	0.33^{ab}	0.34^{a}

Values in a column without the same superscript are significantly different

Table 4.11 summarises the results of the histological examination of kidneys taken from birds. The incidence of abnormality (e.g. lymphocyte infiltration) was low and not different across the weeks.

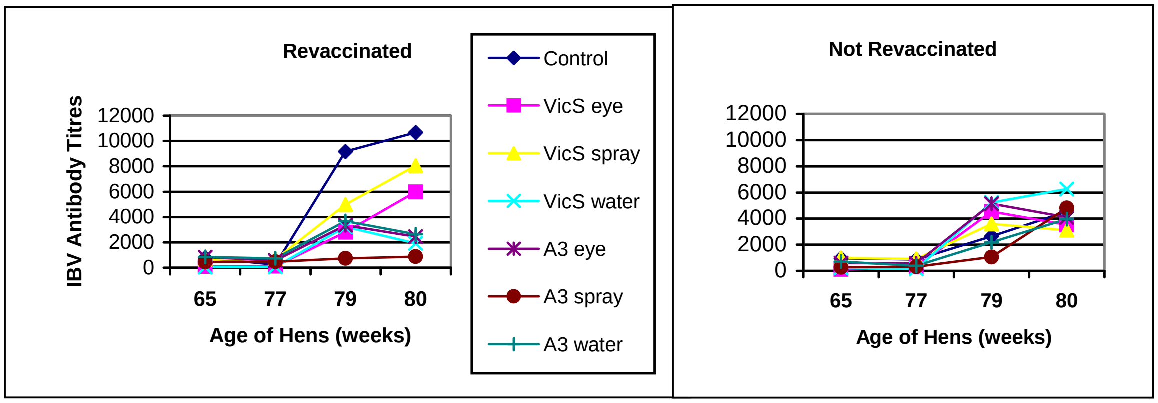
Table 4.11 Histology of left cranial kidneys at weeks post exposure to T strain IBV

Week	Total kidney samples	Positive abnormality	% Abnormality
0	94	6	6.38
1	56	3	5.36
2	56	2	3.57
3	56	3	5.36
4	35	1	2.86
5	35	0	0

4.4.2.4 IBV Antibody Titres
Infectious bronchitis antibody titres increased significantly (P<0.0001) following exposure to T-strain IBV, as shown in Figure 4.1. However, it is apparent from Figure 4.1 that some initial vaccination treatment groups responded to a greater extent in response to challenge than did others. For example, in the birds that were revaccinated every 8 weeks during lay, the A3 spray birds responded weakly whereas the control birds responded strongly.

There was no statistically significant effect of initial vaccination treatment, regular revaccination during lay, or timing of moult in relation to revaccination, on IBV antibody titres.

Figure 4.1 IBV antibody titres in relation to challenge with T-strain IBV



4.5 Discussion and Conclusions

In the present study, up until 56 weeks of age, it was found that vaccination treatment, including regular revaccination for IB, affected egg production and regular revaccination had some deleterious effects on egg shell quality (Sulaiman *et al*., 2002). For older birds, revaccination following an induced moult resulted in significantly better egg shell quality than revaccination prior to an induced moult. This finding was consistent when birds were compared at the same ages or at the same duration following moult. Shell breaking strength was better late in lay for birds that had been vaccinated initially with the A3 strain.

Regular revaccination during lay resulted in higher excreta moisture following revaccination late in lay. However, the higher excreta moisture found in the birds which were moulted following revaccination would be due mainly to the fact that those birds were consuming whole grain barley.

The antibody titres were relatively low for all groups and were affected only by the revaccination at 62 weeks which resulted in slightly elevated titres in all treatment groups.

When birds were challenged with T-strain IBV at 77 weeks of age, the main effect was an increase in the incidence of abnormal eggs and a large rise in IBV antibody titres in most treatment groups. There were few effects on kidney histology.

Results suggest that there is little advantage in regularly revaccinating laying hens for IB virus, provided that they have received appropriate vaccination during the rearing phase. IB vaccination in moulted birds is best given after the moult.

5. Trial 2: Effects of bird strain and vaccine strain in providing protection against challenge with T-strain infectious bronchitis virus (IBV) in laying hens – challenge at 25 weeks

5.1 Introduction

The results of Trial 1 indicated that all birds were reasonably well protected against challenge with T-strain IBV at the age of 77 weeks. However, in that trial, all birds had been revaccinated with VicS at 62 weeks of age. In addition, all vaccination groups except the Control Group in Trial 1 had received both VicS and A3 vaccine strains during the rearing period.

Trial 2 incorporated vaccination groups that received either one or other vaccine strain, or a combination. In addition, the birds in Trial 2 were not revaccinated beyond 14 weeks of age and were then challenged with T-strain IBV at three different ages. This experiment addressed the question of whether or not vaccination close to the onset of lay has a deleterious effect on bird performance.

5.2 Objectives

The objectives of the 25 week challenge of Trial 2 were:

- To assess the effects of IBV challenge in early lay
- To assess the relative effects of administering the third IBV vaccination during rearing at either 14 weeks (prior to the onset of lay) or at 18 weeks (5% lay).

5.3 Methodology

5.3.1 The Trial 2 Flock

Day-old commercial laying pullets (900 birds) were transferred to the isolation pens at the University of New England. There were four strains, Isa Brown (250), HiSex Brown (250 birds), HyLine Brown (200 birds), Hyline Grey (200 birds). On arrival 100 birds (50 Isa Brown and 50 HiSex) were kept separate for the first challenge experiment. The remaining birds were divided into two groups, before being vaccinated by coarse spray against IBV, half received the A3 vaccine strain and the other half VicS strain. The pullets were then raised on the floor of the isolation pens and received a second IBV vaccination at 4 weeks of age. Birds were vaccinated for Marek's at day-old and AE (avian encephalomyelitis) at 12 weeks of age by eyedropping one in 5 birds.

The vaccination groups were: A3 strain at day-old, 4 weeks and 14 weeks (AAA); A3 strain at day-old, VicS at 4 weeks and A3 at 14 weeks (AVA); VicS at day-old, 4 weeks and 14 weeks (VVV); VicS at day-old, A3 at 4 weeks and VicS at 14 weeks (VAV).

Birds received broiler starter for 2.5 weeks, pullet starter ration to 4.5 weeks of age, pullet grower to 14 weeks, prelayer (grower with additional calcium and yolk colour) until 5% production (19 weeks). At that stage, birds received SuperAll layer crumbles or mash (Ridley AgriProducts).

5.3.2 The Birds for Challenge at 25 weeks

The 100 birds used for the challenge experiment at 25 weeks were vaccinated at day-old, half the birds with A3 and half with VicS. At 4 weeks, half of the birds that received A3 at day-old received A3 and the other half VicS. Of the birds that received VicS at day-old old, all received VicS at 4 weeks of age. At 14 weeks of age the treatment groups were divided again with half being vaccinated at this time and the other half when they had reached 5% lay (18 weeks of age). The 14/18 week (third) vaccination was the same vaccine strain as the birds received at 4 weeks.

At 20 weeks of age, the 100 pullets were relocated to a separate isolation shed. At 25 weeks of age, these birds were exposed to T-strain IBV. Every second bird was eye-dropped with a dose calculated to average 10^4 median ciliostatic doses (CD_{50}) of virus per bird.

Blood samples were taken from the same ten birds in each treatment group at 2 weeks prior to and then 2, 3, 4 and 5 weeks after exposure. The blood samples were analysed for plasma electrolytes (Na^+, K^+ and Ca^{++}) and haematocrit. 80 eggs were collected (20 per treatment group) and analysed at 1 week before, then 1, 2, 3, 4, 5 and 10 weeks after challenge. Eggs were tested for both internal and external quality measures. At 2 weeks post challenge 2 birds per group were euthanased and both kidneys removed, weighed and calculated as a percentage of body weight. The kidneys, trachea and oviduct were removed for histological analysis at a later date. Daily egg production records were kept for all groups. External visual classification of shell defects was carried out daily recording all abnormalities. IBV antibody titres were measured in blood samples taken from 5 birds both before challenge and 3 weeks after challenge. IBV antibody titres were measured at a commercial facility using both IDEXX and TropBio ELISA kits.

Analysis of Variance was used to test the effect of challenge on each of the egg quality, blood analysis and kidney weight parameters measured. Claims of statistical significance were based on $P<0.05$. Fishers protected LSD was used to distinguish between means when significant effects were seen.

5.4 Detailed Results

5.4.1 Body Weight

Body weights of birds during the rearing period are shown in Table 5.1. The only ages at which there were significant differences among the vaccination groups were 2 and 3 weeks of age when body weight was higher in the VAV group. Patterns of early growth were different among the strains (Table 5.2).

Table 5.1 Body weights of birds during rearing in relation to initial vaccination treatment

	2 Wks	3 Wks	4 Wks	5 Wks	6 Wks	7 Wks	16 Wks	18 Wks
AAA	107.20^b 1.72	161.58^c 3.11	265.35^a 4.08	349.65^a 4.87	457.03^a 8.47	592.50^a 7.84	1585.00^a 21.05	1698.75^a 25.54
AVA	104.10^b 2.03	173.25^b 3.72	271.28^a 4.77	360.68^a 5.58	468.98^a 8.72	588.65^a 8.18	1542.50^a 21.75	1668.75^a 26.05
VVV	108.35^b 2.07	170.03^{bc} 3.64	258.75^a 5.13	360.70^a 6.31	467.25^a 8.17	598.23^a 10.51	1568.75^a 24.46	1750.00^a 29.85
VAV	115.58^a 2.14	183.20^a 2.66	266.13^a 3.99	357.68^a 4.95	465.15^a 6.96	581.70^a 8.45	1512.50^a 19.84	1696.25^a 23.91
P-value	0.0008	0.0001	0.2784	0.4363	0.7350	0.6029	0.1011	0.1814

Mean ± S.E. Values in columns with no common superscript differ significantly
A is A3 strain vaccine, V is VicS strain vaccine

Table 5.2 Body weights of birds during rearing in relation to strain of bird

	2 Wks	3 Wks	4 Wks	5 Wks	6 Wks	7 Wks	16 Wks	18 Wks
Isa Brown	110.4[a] 1.9	166.9[bc] 3.1	262.3[ab] 4.5	348.6[b] 5.3	464.8[a] 9.1	592.0[ab] 8.5	1588.8[a] 17.8	1751.3[a] 23.0
Hyline Brown	111.2[a] 2.0	175.3[b] 3.3	272.1[a] 4.6	366.8[a] 6.0	473.3[a] 7.7	609.9[a] 9.1	1612.5[a] 19.0	1747.5[a] 24.2
Hyline Grey	115.4[a] 1.6	185.2[a] 2.7	274.4[a] 3.7	363.3[ab] 5.0	464.5[a] 6.2	582.9[b] 8.0	1437.5[b] 20.0	1566.3[b] 22.45
HiSex	98.3[b] 1.9	160.7[c] 3.5	252.8[b] 4.5	350.1[b] 5.1	455.9[a] 9.0	576.4[b] 8.8	1570.0[a] 20.9	1748.8[a] 24.9
P-value	<0.0001	<0.0001	0.0021	0.0342	0.5105	0.0389	<0.0001	<0.0001

Mean ± S.E. Values in columns with no common superscript differ significantly

5.4.2 Egg Production and External egg classification

There were no significant effects of challenge with T-strain on egg production when the challenged birds were in early lay (Figure 5.1, Table 5.3). Total egg abnormalities increased after exposure to T-strain IBV until 2 weeks post challenge. The highest daily percentage of eggs laid that had a visually obvious abnormality was 57%, recorded at 13 days after exposure to the virus. By the eighth week after IBV challenge, the percentage of abnormal eggs had returned to pre-challenge levels. The incidence of one particular recorded abnormality, fine longitudinal wrinkles, increased from 0 to over 25% of the eggs produced (Figure 5.2).

Figure 5.1 Production of flock that was challenged in early lay with T-strain Infectious Bronchitis. The arrow indicates the time at which the challenge occurred

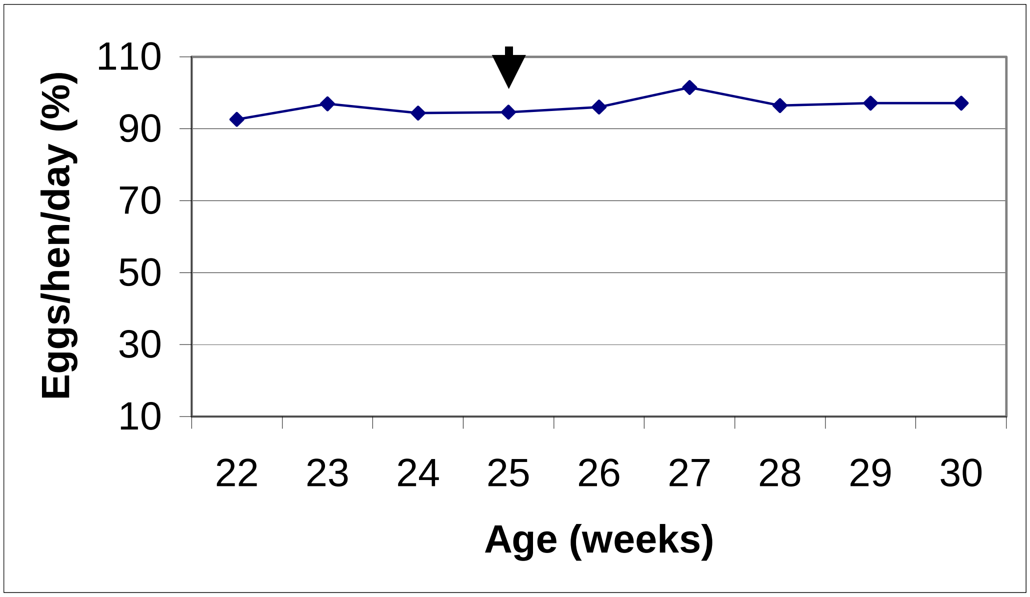
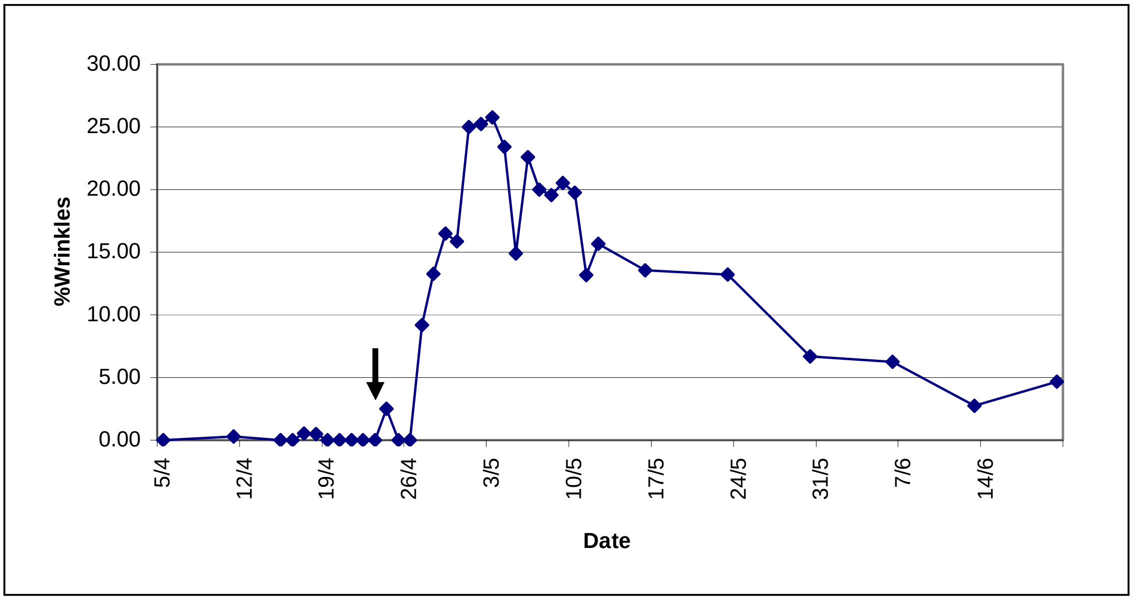


Table 5.3 Production from 22 to 30 weeks

3rd vacc	Wk 22	Wk 23	Wk 24	Wk 25	Wk 26	Wk 27	Wk 28	Wk 29	Wk 30	P value
Early	85.2 ±11.5	96.9 ±2.3	95.3 ±2.2	93.7 ±2.6	94.7 ±0.9	101.8 ±0.9	96.1 ±2.0	97.9 ±1.7	96.8 ±1.4	0.2306
Late	100.0 ±5.3	97.1 ±1.1	93.4 ±2.1	95.5 ±1.8	97.4 ±0.7	101.1 ±0.9	96.8 ±0.7	96.5 ±1.7	97.5 ±0.8	
Total	92.6 ±6.5	97.0 ±1.2	94.4 ±1.5	94.6 ±1.5	96.0 ±0.7	101.4 ±0.6	96.4 ±1.0	97.2 ±1.1	97.1 ±0.8	0.3831

Mean ± S.E.

Figure 5.2 Percent of eggs produced that were wrinkled. The arrow indicates the time at which the birds were challenged just before 25 weeks of age.



5.4.3 Egg Quality

Egg quality during the experiment is shown in Table 5.4. Yolk colour and deformation were not affected by infection with T-strain IBV. Egg weight and shell weight increased significantly and steadily across the challenge period. Shell thickness remained constant during weeks 1-3 after challenge with T-strain, and then began to increase. At one-week post infection, breaking strength of the eggshell was significantly reduced, but had returned to pre exposure levels 10 weeks after challenge. Shell reflectivity was significantly increased by challenge with T-strain and it remained high. There was a significant drop in Haugh units measured at 1 week after challenge. Egg weight and Haugh units were significantly lower for the birds that were vaccinated late at 18 weeks of age. Shell reflectivity and percentage shell were higher for this treatment group.

Table 5.4 Effect of T-strain IBV challenge on egg quality parameters

	Before	After Exposure						
	1 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	10 Weeks	P Value
Haugh units	^{a}103.64 ± 4.21	^{d}96.73 ± 4.12	^{b}99.95 ± 4.85	^{c}98.48 ± 3.91	^{d}95.99 ± 4.25	^{d}96.58 ± 5.29	^{e}94.51 ± 5.80	<.0001
Reflectivity (%)	^{c}23.46 ± 3.73	^{b}28.73 ± 4.69	^{b}28.71 ± 5.81	^{a}30.53 ± 5.35	^{ab}29.40 ± 4.75	^{ab}29.03 ± 5.16	^{a}30.42 ± 5.81	<.0001
Shell weight. (g)	^{d}5.56 ± .41	^{c}5.76 ± .46	^{c}5.86 ± .44	^{c}5.84 ± .43	^{b}6.02 ± .33	^{ab}6.13 ± .47	^{a}6.26 ± .47	<.0001
Shell thickness (μm)	^{b}430.65 ± 22.42	^{c}427.23 ±23.39	^{bc}430.42 ± 23.82	^{c}424.07 ± 19.50	^{ab}436.89 ± 22.15	^{b}435.91 ± 22.61	^{a}443.33 ±25.95	<.0001
Breaking strength (N)	^{ab}44.81 ± 7.38	^{d}40.56 ± 6.90	^{bc}43.24 ± 9.33	^{cd}41.71 ± 7.79	^{bcd}42.33 ± 7.75	^{cd}41.45 ± 9.37	^{a}45.87 ± 8.11	0.0002
Deformation (μm)	297.50^{a} ±10.64	264.25^{b} ±12.14	270.25^{b} ±7.26	278.57^{ab} ±10.84	276.99^{ab} ±9.90	264.62^{b} ±6.33	264.13^{b} ±7.53	0.1360
Egg weight (g)	55.16^{e} ±0.40	56.83^{d} ±0.39	57.90^{d} ±0.43	59.18^{c} ±0.45	60.46^{b} ±0.50	61.86^{a} ±0.52	62.19^{a} ±0.43	<0.0001
Albumen height (mm)	10.73^{a} ±0.12	9.32^{de} ±0.10	10.10^{b} ±0.13	9.81^{bc} ±0.10	9.34^{d} ±0.10	9.54^{cde} ±0.13	9.15^{e} ±0.13	<0.0001
Yolk colour	10.90^{c} ±0.11	10.94^{c} ±0.11	11.05^{c} ±0.10	10.92^{c} ±0.10	11.85^{a} ±0.09	11.00^{c} ±0.03	11.48^{b} ±0.09	<0.0001
% Shell	10.09^{abc} ±0.07	10.15^{a} ±0.07	10.14^{ab} ±0.07	9.88^{e} ±0.06	9.95^{bcde} ±0.09	9.93^{cde} ±0.07	10.07^{abc} ±0.08	0.0331

Mean ± S.E. Means in rows with no common superscript differ significantly ($P<0.05$)

Table 5.5 shows the difference between early vaccination at 14 weeks of age and late vaccination at 18 weeks of age on egg and egg shell quality. Shell colour was darker in birds vaccinated early and egg weight, shell deformation and Haugh Units were higher. However, percentage shell was higher for the birds vaccinated at 18 weeks.

Table 5.5 Effect of timing of third vaccination on egg quality parameters under the stress of challenge.

	Early	Late	P Value
Haugh units	^{a}99.29 ± 5.48	^{b}96.75 ± 5.14	<.0001
Reflectivity (%)	^{a}28.00 ± 6.10	^{b}29.17 ± 4.86	0.0086
Egg weight (g)	^{a}59.92 ± 4.63	^{b}58.21 ± 4.47	<.0001
% Shell	^{a}9.92 ± 0.64	^{b}10.15 ± 0.66	<.0001
Deformation (Mm)	^{a}275.39 ±4.83	^{a}272.10 ±5.31	0.6175
Breaking Strength (N)	^{a}43.06 ±0.50	^{a}42.68 ±0.51	0.5772
Shell weight (g)	^{a}5.94 ±0.03	^{a}5.90 ±0.03	0.2563
Yolk colour	^{a}11.14 ±0.06	^{a}11.17 ±0.05	0.8202
Shell thickness (µm)	^{a}430.89 ±1.36	^{a}434.33 ±1.48	0.0923
Albumen height (mm)	^{a}10.04 ±0.07	^{a}9.40 ±0.06	<0.0001

Means in rows with no common superscript differ significantly (P<0.05)

5.4.4 Kidney weights

At two weeks after exposure to T-strain IBV, kidney weight as a percentage of body weight was not significantly different between treatment groups (Table 5.6).

Table 5.6 Kidney weights of birds in relation to timing of third vaccination

	Early	Late	P-value
Total kidney weight as % body weight	^{a}0.70 ±0.02	^{a}0.68 ±0.02	0.2144
R/L kidney weight ratio	^{a}0.97 ±0.02	^{a}1.01 ±0.01	0.0742

Means ± SEM. Means in rows with different superscripts are significantly different.

5.4.5 Blood

Blood analysis results are shown in Table 5.7. There were no significant differences resulting from vaccine strain used for first vaccination or timing of the third vaccination. However, there were changes in relation to time. Haematocrit increased at 2 weeks, declined, then increased steadily. Plasma sodium, calcium, potassium, chloride and osmolality were significantly lower at 2 weeks post-challenge than levels 2 weeks prior to challenge. Potassium levels in the blood were also lower at 4 weeks after exposure to T-strain IBV.

Table 5.7 Effect of challenge on blood analysis

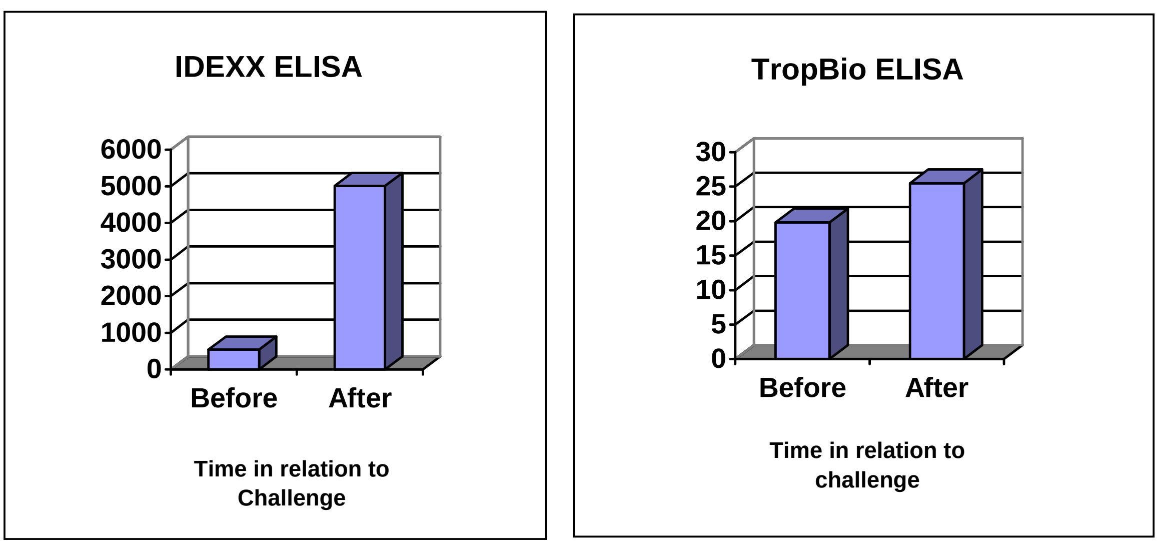
	Before	After Exposure				
	2 weeks	2 weeks	3 weeks	4 weeks	5 weeks	P Value
Haematocrit %	[c]20.95 ± 2.11	[b]27.89 ± 2.14	[bc]27.24 ± 1.94	[ab]28.01 ± 1.40	[a]28.81 ± 2.16	0.0006
[Ca] mM	[b]1.77 ± 0.09	[c]1.69 ± 0.10	[b]1.75 ± 0.10	[a]1.82 ± 0.12	[a]1.81 ± 0.11	<0.0001
[Na] mM	[a]151.0 ± 2.4	[b]142.6 ± 3.2	[a]491.7 ± 2.3	[a]149.7 ± 3.2	[a]150.5 ± 4.1	<0.0001
[K] mM	[a]5.71 ± .29	[c]5.16 ± .32	[a]5.70 ± .34	[d]5.00 ± .27	[b]5.43 ± .38	<0.0001
Osmolality mmol/kg	[ab]312.8 1.24	[c]297.9 1.47	[ab]312.2 1.15	[b]310.0 1.54	[c]314.7 2.27	<0.0001
[Cl] mM	[c]111.5 0.82	[d]107.3 1.00	[a]117.7 0.94	[ab]115.7 1.11	[bc]114.0 1.23	<0.0001

Means ± S.E. Means in rows with different superscripts are significantly different

5.4.6 IBV Antibody Titres

The IBV antibody titres measured prior to challenge and then 3 weeks following challenge are shown in Figure 5.3. The mean titre increased, as measured by both IDEXX and TropBio ELISA kits. However, the difference was statistically significantly different for IDEXX but not TropBio.

Figure 5.3. IBV antibody titres before and 3 weeks after challenge



5.5 Discussion and Conclusions

The dramatic increase in the number of abnormal eggs was due largely to an increased incidence of eggs with wrinkled shells. These wrinkles were not the large undulations that are commonly referred to as an “IB egg” but ran the length of the egg and were no greater than 1 mm in width. Wrinkled eggs are an indication that inflammation of the tissues of the oviduct, which occurs during T-strain infection, results in inadequate “plumping” of the egg and therefore a wrinkled appearance of the finished shell.

A steady increase in both egg weight and shell weight would be expected at this stage of lay. However, both quality parameters, particularly shell weight, appear to have experienced a delay in rate of increase across weeks 1, 2 and 3 post-challenge. This slight, but significant ($P<0.0001$), effect corresponds to the levelling off of the normally expected increase in shell thickness during this period. Shell breaking strength decreased significantly at 1 week after infection and remained low but had returned to previous strength 10 weeks after challenge. Shell reflectivity was markedly increased (shell colour was paler) by exposure to T-strain and this effect was sustained. Paler shell colour may be due to extra calcium deposits laid down in the shell gland on the outside of an otherwise completed egg, another indication of a malfunctioning oviduct. Across the weeks after infection with T-strain IBV the Haugh units were seen to trend downward slowly, as would be expected at this stage of lay. However, at one week after challenge there was significant drop in albumen height and therefore Haugh units. This drop in Haugh unit was expected, as IBV is known to cause a thinning of the albumen. However, the investigator noted that there was a decline in the quality of the thin albumen in the eggs for two weeks after infection with IBV. The significant effects due to the timing of the birds’ third IBV vaccination (Table 5.5) are decreased egg quality in the late vaccinated birds, compared to those vaccinated for the third time well before lay had commenced. Hence it can be concluded that vaccination with a live IBV vaccine virus at the onset of lay, when the oviduct is rapidly developing, may lead to damage of the oviduct and therefore a decrease in the quality of eggs produced. T-strain IBV is known as a nephropathogenic strain of the virus that causes a marked nephritis. While damage to the kidneys was not apparent in the kidneys of the euthanased birds, the changes in the plasma electrolyte levels are probably due to excess excretion of electrolytes.

In conclusion, exposure to T-strain IBV at 25 weeks of age had deleterious effects on egg quality and blood electrolytes, particularly if the birds had received their third IBV vaccination at the onset of lay.

6. Trial 2: Effects of bird strain and vaccine strain in providing protection against challenge with T-strain infectious bronchitis virus (IBV) in laying hens – challenge at 41 weeks

6.1 Introduction
This stage of Trial 2 was a challenge of the birds by two different challenge viruses, at peak lay. The standard challenge virus remained the nephropathogenic T-strain IBV. However, in order to test for protection against other strains, the respiratory strain N1/88 was also used.

6.2 Objectives
The aims of this study were to assess the effects of challenge, at peak lay (41 weeks of age), of birds that had received different vaccination protocols during lay but were not revaccinated since the third vaccination at 14 weeks of age. Two challenge viruses were used: T-strain IBV which is known to be nephropathogenic and N1/88 which appears to target the respiratory system as well as the kidneys.

6.3 Methodology
The birds were reared as described in Chapter 5. A subsample of birds was transferred from the large isolation shed on campus to smaller isolation sheds. There were 4 strains of birds, 4 initial vaccination treatments (AAA, AVA, VVV, VAV) and 3 challenge treatment groups (control, T-strain and N1/88). There was a total of 256 birds in the viral challenge groups (128 per virus strain) and 128 control birds that remained in a large isolation shed. All birds were housed 3 to a cage.

The challenge viruses were purchased from Dr. Jagoda Ignjatovic of the CSIRO Australian Animal Health Laboratory in Geelong. The T virus was from the same stock had been shown previously to cause mortality and kidney damage in unvaccinated cockerels (Sulaiman et al., 2001a,b).

Production was measured continuously throughout the experiment. Egg and egg shell quality were measured immediately prior to the challenge and at weekly intervals for 5 weeks postchallenge. For each egg collection, 10 eggs were taken from each of the 48 experimental groups.

Excreta moisture was measured prior to challenge and weekly for 4 weeks postchallenge in the two challenge groups. On each sampling occasion, excreta were collected from 3 replicates (each of 3 birds) for each of the experimental groups.

Blood samples were taken from 5 birds per challenge group prior to the challenge and then at weekly intervals for 6 weeks postchallenge. Kidneys were taken from a total of 64 birds, 2 birds per challenge group, for histological examination. IBV antibody titres were measured in samples of plasma prior to and three weeks following challenge, using a TropBio IBV Antibody ELISA kit. IBV antibody data are presented for the Isa Brown birds, only, owing to time constraints.

6.4 Detailed Results

6.4.1 Production

The "Before Challenge" period was 3 weeks prior to the challenge, "During Challenge" was a period of 3 weeks from the administration of the challenge virus, "After Challenge" was a subsequent period of 3 weeks. There were significant main effects of time period of the experiment ($P<0.0001$), exposure to challenge virus ($P=0.0178$) and strain of bird ($P<0.0001$). The production of the different vaccination treatment groups during the challenge at 41 weeks of age is shown in Table 6.1. There was no significant effect of vaccination treatment group on production at any stage of the experiment

Table 6.1 Effect of vaccination treatment group and stage of challenge on egg production.

Vacc group	A3/A3/A3	A3/VicS/A3	VicS/VicS/VicS	VicsS/A3/VicS
Before Challenge	84.73 ±1.39	85.01 ±1.45	86.69 ±1.50	86.71 ±1.50
During Challenge	86.32 ±1.51	86.73 ±1.69	85.14 ±1.55	89.04 ±1.33
After Challenge	89.59 ±1.57	91.35 ±1.03	88.71 ±1.19	89.86 ±1.36
All Time Periods	86.88 ±0.87	87.72 ±0.83	86.85 ±0.82	88.54 ±0.81

Mean ± S.E.

There were significant differences among the 4 strains at all stages of the challenge experiment (Table 6.2). Across all three time periods the significant differences in production follow a similar pattern, with Hyline Greys being consistently the lowest producers and Isa Brown the highest, and the Hyline Browns and HiSex intermediate.

Table 6.2 Effect of genetic strain and stage of challenge on egg production level

Strain	Isa	Hyline Brown	Hyline Grey	HiSex	P Value
Before Challenge	89.55^{a} ±1.21	85.99^{a} ±1.43	81.68^{b} ±1.55	85.91^{a} ±1.42	0.0017
During Challenge	89.90^{a} ±1.34	86.09^{ab} ±1.61	82.55^{b} ±1.66	88.79^{a} ±1.24	0.0024
After Challenge	94.04^{a} ±0.94	90.64^{ab} ±1.01	85.04^{c} ±1.53	89.80^{b} ±1.32	< 0.0001
All Time Periods	91.81^{a} ±0.69	87.59^{bc} ±0.81	83.09^{c} ±0.91	88.17^{b} ±0.78	<0.0001

Mean ± S.E. Data in the same row with the same superscript figure are not significantly different from one another

During challenge the group that was exposed to T-strain IBV had a reduction in egg production that was statistically significant (Table 6.3). However, production was not significantly affected by the N1/88 IBV.

Table 6.3 The effect of challenge virus on egg production

Challenge group	T Strain	N1/88	Control	P Value
Before Challenge	85.38 ±1.35	85.40 ±1.44	86.17 ±1.08	N/S
During Challenge	82.75[b] ±1.60	87.86[a] ±1.39	88.36[a] ±1.04	0.0067
After Challenge	89.71 ±1.28	88.28 ±1.23	90.76 ±0.95	NS
All Time Periods	85.95[b] ±0.85	87.16[ab] ±0.79	88.43[a] ±0.60	0.0464

Mean ± S.E. Data in the same row with the same superscript figure are not significantly different at the P=0.005 level.

6.4.2 Egg and Egg Shell Quality

There was a statistically significant main effect of challenge treatment on egg weight, shell weight, percentage shell, shell thickness, albumen height, Haugh units and yolk colour, as shown in Table 6.4. Shell quality was depressed in the two challenge groups which had lower egg weight and shell weight than the control group. The N1/88 group had the lowest shell thickness. An unexpected result was that albumen quality was higher in the challenge groups than for the control.

Table 6.4 Main effect of challenge group on egg and egg shell quality.

	Control	T-strain	N1/88	P Value
Egg Weight (g)	[a]62.7 ± 0.1	[b]59.7 ± 0.3	[b]60.0 ± 0.4	P<0.0001
Shell Reflectivity (%)	42.1 ± 0.3	42.0 ± 0.4	41.8 ± 0.4	NS
Breaking Strength (N)	42.0 ± 0.3	42.1 ± 0.2	41.9 ± 0.2	NS
Deformation (μm)	265.1 ± 1.5	264.6 ± 2.2	270.0 ± 2.3	NS
Shell weight (g)	[a]6.07 ± 0.01	[b]5.95 ± 0.02	[b]5.97 ± 0.02	<0.0001
% Shell	[ab]9.68 ± 0.02	[a]9.72 ± 0.03	[b]9.64 ± 0.03	0.0366
Shell Thickness (μm)	[a]425.0 ± 0.8	[a]424.6 ± 1.0	[b]422.0 ± 1.0	0.0098
Albumen Height (mm)	[c]8.98 ± 0.03	[b]9.07 ± 0.04	[a]9.41 ± 0.04	<0.0001
Haugh Units	[c]93.4 ± 0.1	[b]94.3 ± 0.2	[a]95.8 ± 0.2	<0.0001
Yolk Colour Score	[a]10.9 ± 0.01	[b]10.8 ± 0.02	[b]10.9 ± 0.02	0.0027

Mean ± S.E. Across a row, values with different superscripts are significantly different from one another. NS is not significant.

There was a statistically significant main effect of time during the challenge experiment on egg weight, shell reflectivity, shell breaking strength, deformation, shell weight, percentage shell, shell thickness, albumen height, Haugh Units and yolk colour as shown in Table 6.4. The interactions between time during the challenge experiment and the challenge group are shown in Tables D1 and D2 in Appendix D. Some of the changes that occurred in egg shell quality and egg internal quality over the time course of the experiment are related to increasing hen age. Egg weight was lower for both challenge groups at 1, 3 and 4 weeks postchallenge and at 2 and 5 weeks for the T group. Deformation was significantly reduced for the T group for several weeks following challenge.

There was a statistically significant main effect of initial vaccination group on shell reflectivity and deformation, only (Table 6.6). Significant interactions between initial vaccination group and challenge group are shown in Tables E3 and E4 in Appendix D.

There was a significant main effect of strain of bird on egg weight, reflectivity, shell breaking strength, deformation, shell weight, percentage shell, shell thickness, albumen height, and yolk colour (Table 6.7). Egg shell quality was best for the Isa Brown and HiSex Brown and albumen quality best for the HyLine Brown and HyLine Grey.

Table 6.5 Main effect of stage of challenge on egg and egg shell quality.

	Before	After Challenge					
		1 Wk	2 Wks	3 Wks	4 Wks	5 Wks	P Value
Egg Weight (g)	^{a}61.6 ± 0.3	^{c}60.0 ± 0.5	^{a}62.0 ± 0.2	^{bc}60.7 ± 0.4	^{ab}61.4 ± 0.4	^{a}61.9 ± 0.3	<0.0001
Shell Reflectivity (%)	^{d}40.7 ± 0.5	^{ac}42.2 ± 0.5	^{bc}41.9 ± 0.5	^{c}41.7 ± 0.5	^{ab}42.5 ± 0.5	^{a}42.9 ± 0.5	<0.0001
Breaking Strength (N)	^{c}40.6 ± 0.3	^{b}41.9 ± 0.3	^{b}42.3 ± 0.3	^{bc}41.5 ± 0.3	^{a}43.7 ± 0.7	^{b}42.0 ± 0.3	<0.0001
Deformation (μm)	^{a}275.3 ± 3.0	^{ab}271.2 ± 3.3	^{c}263.2 ± 2.7	^{c}262.6 ± 2.7	^{bc}264.9 ± 2.0	^{c}259.7 ± 2.0	0.0064
Shell weight (g)	^{e}5.78 ± 0.03	^{d}5.97 ± 0.02	^{a}6.24 ± 0.02	^{bc}6.04 ± 0.02	^{b}6.06 ± 0.02	^{cd}6.00 ± 0.02	<0.001
% Shell	^{c}9.34 ± 0.04	^{b}9.65 ± 0.03	^{a}10.05 ± 0.03	^{b}9.73 ± 0.03	^{bc}9.72 ± 0.03	^{c}9.61 ± 0.03	<0.0001
Shell Thickness (μm)	^{c}416.0 ± 1.5	^{a}428.0 ± 1.4	^{a}430.3 ± 0.2	^{b}424.4 ± 1.3	^{b}424.4 ± 1.2	^{b}421.9 ± 1.2	<0.0001
Albumen Height (mm)	^{a}9.34 ± 0.04	^{b}9.13 ± 0.05	^{c}8.91 ± 0.05	^{c}8.95 ± 0.05	^{b}9.12 ± 0.05	^{b}9.21 ± 0.05	<0.0001
Haugh Units	^{a}95.5 ± 0.2	^{b}94.5 ± 0.2	^{c}93.2 ± 0.2	^{c}93.4 ± 0.3	^{b}94.2 ± 0.2	^{b}94.6 ± 0.3	<0.0001
Yolk Colour Score	^{a}11.2 ± 0.03	^{b}11.0 ± 0.03	^{e}10.6 ± 0.03	^{d}10.7 ± 0.03	^{c}10.9 ± 0.03	^{bc}11.0 ± 0.02	<0.0001

Mean ± S.E. Across a row, values with different superscripts are significantly different from one another. NS is not significant.

Table 6.6 Egg shell quality for the different vaccination groups

	AAA	AVA	VVV	VAV	P-value
Egg Weight (g)	61.4 ± 0.3	61.4 ± 0.3	61.2 ± 0.3	61.0 ± 0.3	NS
Shell Reflectivity (%)	^{b}41.7 ± 0.4	^{ab}42.0 ± 0.4	^{ab}41.9 ± 0.4	^{a}42.3 ± 0.4	0.0474
Break Strength (N)	42.2 ± 0.5	41.6 ± 0.2	42.7 ± 0.2	41.5 ± 0.3	NS
Deformation (μm)	^{b}264.2 ± 2.3	^{b}262.6 ± 1.8	^{a}272.6 ± 2.4	^{b}265.4 ± 21	0.0068
Shell Weight (g)	6.00 ± 0.02	6.01 ± 0.02	6.06 ± 0.02	5.98 ± 0.02	NS
% Shell	9.68 ± 0.03	9.62 ± 0.03	9.75 ± 0.03	9.68 ± 0.03	NS
Shell Thickness (mm)	424.2 ± 1.0	422.7 ± 1.1	426.9 ± 1.0	422.8 ± 1.2	NS
Albumen Height (mm)	9.15 ± 0.04	9.11 ± 0.04	9.09 ± 0.04	9.08 ± 0.04	NS
Haugh Units	94.5 ± 0.2	94.1 ± 0.2	94.0 ± 0.2	94.2 ± 0.2	NS
Yolk Colour	10.9 ± 0.02	10.9 ± 0.02	10.9 ± 0.02	10.9 ± 0.02	NS

Mean ± S.E. Across a row, values with different superscripts are significantly different from one another. NS is not significant.

Table 6.7 Egg quality and genetic strain.

	Isa Brown	Hyline Brown	Hyline Grey	HiSex	P-Value
Egg Weight (g)	62.48^{a} ±0.27	61.77^{ab} ±0.31	59.39^{c} ±0.29	61.34^{b} ±0.26	<0.0001
Shell Reflectivity (%)	35.56^{c} 0.22	36.59^{b} 0.18	60.84^{a} 0.28	35.08^{c} 0.24	<0.0001
Breaking Strength (N)	42.31^{b} ±0.24	40.55^{c} ±0.21	40.52^{c} ±0.20	44.59^{a} ±0.49	<0.0001
Deformation (mm)	257.33^{c} ±1.98	265.20^{b} ±2.06	267.41^{b} ±2.28	274.70^{a} ±2.24	<0.0001
Shell Weight (g)	6.37^{a} ±0.02	5.93^{c} ±0.02	5.63^{d} ±0.02	6.13^{b} ±0.02	<0.0001
% Shell	10.07^{a} ±0.03	9.43^{c} ±0.03	9.33^{d} ±0.02	9.88^{b} ±0.03	<0.0001
Shell Thickness (μm)	445.5^{a} ±0.93	414.8^{c} ±0.94	399.6^{d} ±0.80	436.4^{b} ±0.93	<0.0001
Albumen Height (mm)	8.56^{c} 0.04	9.49^{a} 0.04	9.51^{a} 0.03	8.88^{b} 0.04	<0.0001
Haugh Units	91.05^{d} ±0.22	95.97^{b} ±0.17	96.69^{a} ±0.15	93.22^{c} ±0.17	<0.0001
Yolk Colour (Roche scale)	11.03^{a} ±0.02	11.05^{a} ±0.02	10.55^{c} ±0.02	10.94^{b} ±0.02	<0.0001

Mean ± S.E. P values are for the effect of bird strain.
Values in a row with different superscripts are significantly different from one another

6.4.3 Manure Moisture

Manure moisture varied over time increasing to 2 weeks postchallenge and then decreasing (Table 6.8). There were also strain differences (P<0.0001) (Table 6.9) and differences among the initial vaccination groups (P=0.0007)(Table 6.10).

Table 6.8 Manure moisture of the three experimental groups over time.

	Before	After Challenge				
		1 Week	2 Weeks	3 Weeks	4 Weeks	P-Value
T-Strain	72.88^{c} ±0.82	77.07^{a} ±0.43	78.90^{a} ±0.50	74.26^{b} ±0.81	73.88^{b} ±1.14	<0.0001
N1/88	71.53^{b} ±1.26	75.78^{a} ±0.41	77.51^{a} ±0.41	73.66^{b} ±0.52	73.77^{b} ±0.44	<0.0001
Control	62.94^{b} ±1.39	69.51^{a} ±0.95	71.67^{a} ±0.80	59.44^{b} ±3.65	67.18^{ab} ±0.85	<0.0001
All Groups	72.2^{d} 0.8	76.4^{b} ±0.3	78.2^{a} ±0.3	74.0^{c} ±0.5	73.8^{c} ±0.6	<0.0001

Mean ± S.E. Means in rows with different superscripts are statistically different (P<0.05).

Table 6.9 Manure moisture of the four strains over time

	Before	After Challenge				
		1 Week	2 Weeks	3 Weeks	4 Weeks	P-Value
Isa Brown	70.53^{d} ±1.42	75.37^{ab} ±0.58	77.62^{a} ±0.89	72.44^{cd} ±0.68	73.87^{bc} ±0.54	<0.0001
Hyline Brown	67.82^{c} ±2.05	75.41^{ab} ±0.56	77.02^{a} ±0.42	72.99^{b} ±0.64	72.86^{b} ±0.61	<0.0001
Hyline Gray	72.78^{ab} ±0.78	77.69^{ab} ±0.63	79.83^{a} ±0.53	77.32^{ab} ±1.15	74.44^{b} ±2.25	0.0504
HiSex	73.70^{b} ±0.77	77.22^{a} ±0.55	78.26^{a} ±0.57	73.10^{b} ±0.97	74.15^{b} ±0.56	<0.0001

Mean ± S.E. Means in rows with different superscripts are statistically different (P<0.05).

Table 6.10 Manure moisture of the four initial vaccination treatments over time

	Before	After Challenge				
		1 Week	2 Weeks	3 Weeks	4 Weeks	P-Value
AAA	75.73^{c} ±0.88	76.84^{ab} ±0.56	77.92^{a} ±0.45	74.84^{c} ±0.72	75.22^{bc} ±0.67	0.0089
AVA	68.27^{c} ±2.02	76.99^{a} ±0.64	78.16^{a} ±0.47	75.45^{ab} ±1.32	71.87^{bc} ±2.13	<0.0001
VVV	70.29^{d} ±1.40	75.76^{ab} ±0.62	77.70^{a} ±0.72	72.18^{cd} ±0.71	73.72^{bc} ±0.66	<0.0001
VAV	74.54^{bc} ±0.97	76.11^{b} ±0.62	78.96^{a} ±0.89	73.36^{c} ±0.87	74.50^{bc} ±0.63	<0.0001

Mean ± S.E. Means in rows with different superscripts are statistically different (P<0.05).

6.4.4 Blood Analysis

The effect of challenge on blood parameters over time is shown in Table 6.11. The plasma concentrations of electrolytes generally declined to 2-3 weeks postchallenge before returning to pre-challenge levels. The pattern for haematocrit (Hct) was the reverse with haematocrit being highest at 2 weeks postchallenge.

Table 6.11 Mean blood analysis values for weekly samples for each virus group.

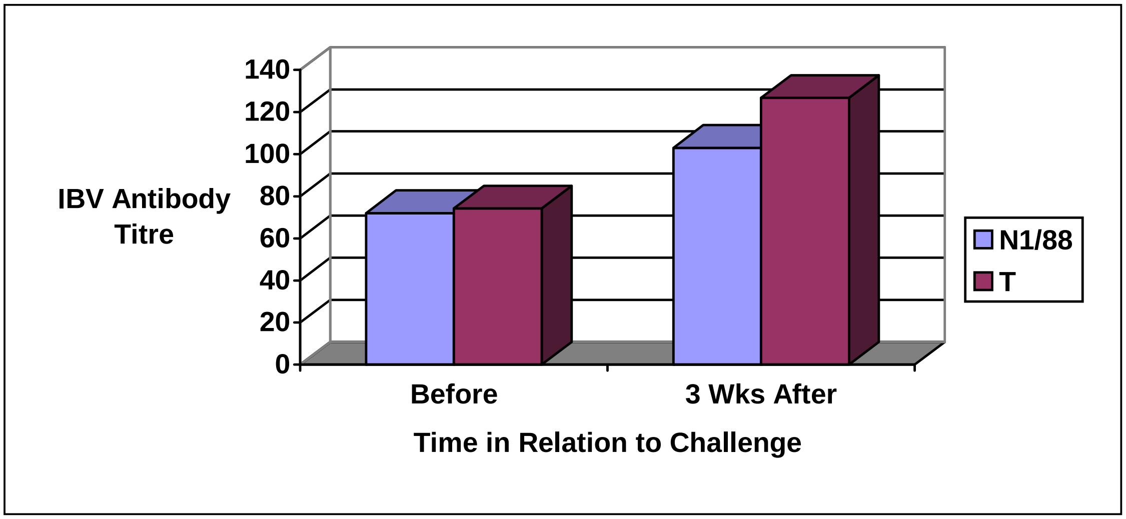
		Before	After Challenge						
			1 Wk	2 Wks	3 Wks	4 Wks	5 Wks	6 Wks	P-value
Na (mm)	N1/88	154.4^{a} ±0.43	147.4^{c} ±0.70	146.6^{cd} ±0.51	145.8^{d} ±0.52	149.9^{b} ±0.31	145.9^{d} ±0.49	150.7^{b} ±0.33	<0.0001
	T	145.4^{d} ±0.55	150.7^{a} ±0.59	143.4^{f} ±0.53	149.5^{b} ±0.36	148.2^{c} ±0.40	145.4^{e} ±0.39	149.6^{ab} ±0.44	<0.0001
K (mm)	N1/88	5.83^{b} ±0.07	5.45^{c} ±0.07	5.51^{c} ±0.06	4.97^{e} ±0.05	5.06^{de} ±0.04	6.22^{a} ±0.06	5.17^{d} ±0.04	<0.0001
	T	5.40^{a} ±0.05	5.67^{b} ±0.07	5.33^{c} ±0.06	5.19^{d} ±0.04	5.19^{d} ±0.04	6.30^{a} ±0.05	5.10^{d} ±0.05	<0.0001
Ca^{++} (mm)	N1/88	1.76^{a} ±0.02	1.62^{c} ±0.02	1.61^{c} ±0.02	1.56^{d} ±0.02	1.70^{b} ±0.02	1.67^{b} ±0.02	1.76^{a} ±0.02	<0.0001
	T	1.57^{b} ±0.02	1.67^{a} ±0.02	1.57^{b} ±0.02	1.69^{a} ±0.01	1.71^{a} ±0.02	1.70^{a} ±0.01	1.71^{a} ±0.02	<0.0001
Hct (%)	N1/88	31.85^{b} ±0.39	31.79^{b} ±0.31	33.02^{a} ±0.34	32.69^{a} ±0.28	31.08^{c} ±0.30	30.90^{cd} ±0.36	30.32^{d} ±0.30	<0.0001
	T	29.66^{c} ±0.34	31.33^{b} ±0.28	33.20^{a} ±0.39	31.29^{b} ±0.28	30.88^{b} ±0.29	31.15^{b} ±0.33	30.74^{b} ±0.39	<0.0001
Osm (mmol/kg)	N1/88	298.9^{d} ±1.03	305.8^{abc} ±1.74	302.6^{bcd} ±1.25	302.1^{cd} ±1.44	306.4^{ab} ±1.77	307.3^{a} ±1.37	304.6^{abc} ±2.04	0.0013
	T	287.2^{e} ±1.23	313.7^{a} ±1.69	302.0^{d} ±2.69	307.9^{b} ±0.85	306.9^{bc} ±1.16	303.2^{cd} ±1.22	308.2^{b} ±1.64	<0.0001
Cl (mm)	N1/88	120.4^{a} ±1.10	116.0^{b} ±1.25	111.6^{d} ±1.12	112.4^{cd} ±1.47	116.3^{b} ±1.00	115.0^{bc} ±1.16	116.0^{b} ±1.55	<0.0001
	T	113.1^{ab} ±1.76	114.5^{a} ±1.07	111.2^{b} ±0.85	115.0^{a} ±0.90	110.5^{b} ±0.72	110.7^{b} ±0.77	113.1^{ab} ±0.95	0.0081
Total Ca (mm)	N1/88	7.49^{bc} ±0.60	9.52^{a} ±0.63	6.86^{c} ±0.40	7.57^{b} ±0.38	8.21^{b} ±0.39	7.52^{bc} ±0.39	8.60^{ab} ±0.61	0.0026
	T	6.34^{d} ±0.72	9.25^{a} ±0.40	6.33^{d} ±0.31	7.13^{bcd} ±0.52	8.38^{abc} ±0.77	6.75^{cd} ±0.67	8.62^{ab} ±0.80	0.0015
P (mm)	N1/88	2.46^{a} ±0.08	2.37^{a} ±0.13	1.80^{cd} ±0.13	1.77^{d} ±0.10	2.19^{ab} ±0.10	2.07^{bc} ±0.09	2.28^{ab} ±0.12	<0.0001
	T	1.99^{b} ±0.11	2.44^{a} ±0.12	2.02^{b} ±0.11	1.68^{c} ±0.15	2.40^{a} ±0.10	1.79^{bc} ±0.13	2.32^{a} ±0.08	<0.0001

Mean ± S.E. Means in rows with different superscripts are statistically different (P<0.05).

6.4.5 IBV Antibody Titres

There was a significant increase in IBV antibody titres 3 weeks postchallenge and a significant interaction between the effects of challenge and the vaccine used (Figure 6.1). There was no significant effect of initial vaccination group on the titre levels.

Figure 6.1 IBV antibody titres in Isa Brown birds immediately before challenge and 3 weeks after challenge with either N1/88 or T-strain virus



6.4.6 Kidney Weights

There were no significant effects of challenge strain (Table 6.12), strain of bird (Table 6.13) or initial vaccine treatment (Table 6.14) on any of the measures of kidney weight.

Table 6.12 Kidney weights of each challenge group

	T	N1/88	P-value
R/L Ratio	1.009[a] ±0.011	1.017[a] ±0.011	0.5423
Kidney weight as % body weight	0.655[a] ±0.02	0.650[a] ±0.013	0.7133

Mean ± S.E. Values in a row with different superscript are significantly different

Table 6.13 Kidney weights of each strain

	Isa Brown	Hyline Brown	Hyline Gray	HiSex	P-value
R/L Ratio	0.999[a] ±0.015	1.013[a] ±0.015	1.035[a] ±0.018	1.007[a] ±0.014	0.4462
Kidney weight as % body weight	0.613[a] ±0.015	0.657[a] ±0.030	0.690[a] ±0.020	0.653[a] ±0.026	.6626

Mean ± S.E. Values in a row with different superscript are significantly different

Table 6.14 Kidney weights of the four initial vaccination treatments

	AAA	AVA	VVV	VAV	P-value
R/L Ratio	1.029[a] ±0.018	1.011[a] ±0.014	1.008[a] ±0.014	1.004[a] ±0.016	0.6139
Kidney weight as % body weight	0.657[a] ±0.016	0.623[a] ±0.022	0.0681[a] ±0.028	0.0651[a] ±0.029	3.915

Mean ± S.E. Values in a row with different superscript are significantly different

6.4.7 Histology

To date the kidneys of all of the Isa Brown birds killed at two weeks after exposure have been examined histologically. Two of the eight birds examined from the T-strain challenged birds showed some histopathology, none of the N1/88 birds showed any damage. The lesions observed in the two birds included medium sized areas of dense lymphocyte infiltration around one or two necrotic tubules in medullary tissue, with a small amount of interstitial oedema. However, this histological damage was found only in one medullary cone per bird and the rest of the kidney showed no damage. It is suggested that the kidney would be capable of fairly normal function and the damage may even be reparable or at the least could be compensated for.

6.5 Discussion and Conclusions

Although the birds challenged at 41 weeks of age were generally well protected by the vaccinations that they had received during rearing, there were some effects of challenge with either T-strain or N1/88 strain IBV. Egg production was reduced during the challenge period in the T group of birds. There were some negative effects of challenge on egg quality, with egg weight and shell weight being lower for the birds challenged with either T or N1/88. Deformation was reduced for the T group, suggesting that the shells had become less elastic, and the birds challenged with N1/88 had the lowest shell thickness. It was expected that one or both of the challenge viruses may have negative effects on albumen quality. However, the opposite response was found, with the challenge groups having higher albumen quality. The reason for this is not known. Manure moisture increased following challenge. However, as there was an increase in the control also, this may have been due to climatic conditions. There were effects of IBV challenge on haematocrit and blood electrolytes with a general pattern of lower electrolyte levels and higher haematocrit 2-3 weeks postchallenge. Such changes could be a reflection of the hydration state of the birds which could, in turn, reflect changes in kidney function. IBV antibody titres, measured by TropBio IBV Antibody ELISA kit showed a statistically significant increase 3 weeks postchallenge. The increase in antibody titre was greater for the T group than the N1/88 group. Effects on the kidneys were relatively minor with no changes in kidney weights and the kidneys from only 2 birds in the T group showed minor histopathology.

The initial vaccination treatment had some effects. Production was lower in the birds that received A3 vaccine at day-old. However, there were few differences among the initial vaccination groups in relation to egg and egg shell quality.

7. Trial 2: Effects of bird strain and vaccine strain in providing protection against challenge with T-strain infectious bronchitis virus (IBV) in laying hens – challenge at 62 weeks

7.1 Introduction

Evidence from the industry suggests that problems with IBV intercurrent infection affecting egg shell quality and egg internal quality are of concern later in lay. This experiment was designed to assess the effects of exposure to a challenge virus towards the end of the laying life of a flock.

7.2 Objectives

The aims of this study were to assess the effects of challenge, at peak lay (62 weeks of age), of birds that had received different vaccination protocols during lay but not revaccinated since the third vaccination at 14 weeks of age. One challenge virus was used, T-strain IBV which is known to be nephropathogenic.

7.3 Methodology

The birds were reared as described in Chapter 5. Birds used in this experiment were divided between two large isolation sheds on campus. There were 4 strains of birds, 4 initial vaccination treatments (AAA, AVA, VVV, VAV) and 2 challenge treatment groups (control and T-strain) – a total of 32 experimental groups. There was a total of 320 birds in the trial, 160 in the challenge group and 160 control birds that remained in a large isolation shed. All birds were housed 2 to a cage.

Production was measured continuously throughout the experiment. Egg and egg shell quality were measured immediately prior to the challenge and at weekly intervals for 6 weeks postchallenge. For each egg collection, 10 eggs were taken from each of the experimental groups.

Excreta moisture was measured prior to challenge and weekly for 4 weeks postchallenge in the two challenge groups. On each sampling occasion, excreta was collected from 3 replicates (each of 3 birds) for each of the experimental groups.

Blood samples were taken from 5 birds per treatment group just prior to challenge and then weekly for 6 weeks following the challenge. IBV antibody titres were measured in samples of plasma prior to and three weeks following challenge, using a TropBio IBV Antibody ELISA kit. IBV antibody data are presented for the Isa Brown birds, only. Kidneys were taken from a total of 64 birds, 2 birds per experimental group, for histological examination.

7.4 Detailed Results

7.4.1 Production

Production before, during and following the challenge is shown in Table 7.1. Before A represents 3 weeks of production before birds were relocated into the experimental groups. Before B is production for 3 weeks after relocation and prior to the challenge. The "During" and "After" periods were each of 3 weeks duration. There was a statistically significant effect of time period ($P<0.0001$) and challenge with T-strain IBV ($P=0.0323$) on production and a significant interaction between these two variables, as shown in Figure 7.1. Production was reduced in the birds that were challenged with T-strain IBV and had not recovered fully by the end of the experiment. This effect was most marked in the birds that had received VicS at day-old, A3 at 4 weeks and VicS at 14 weeks (VAV) (Table 7.3).

Figure 7.1 Effect of challenge with T-strain IBV on production (eggs/100 hens/day)

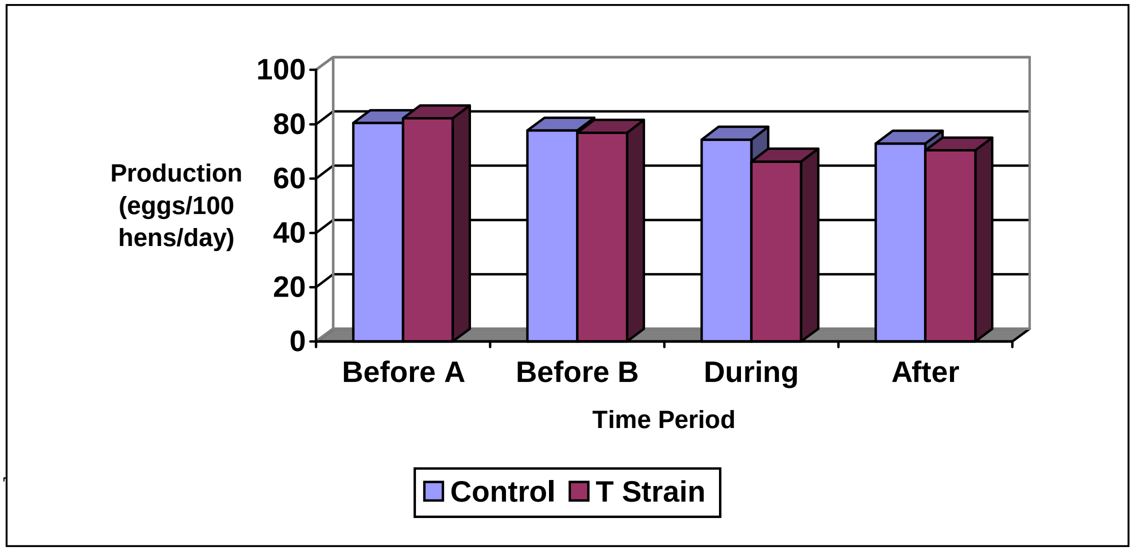


Table 7.1 Egg production before, during and after the challenge

	Before A	Before B	During	After
T-strain	82.17 [a] 1.41	76.93 [a] 1.73	66.19 [b] 2.04	70.35 [a] 1.66
Control	80.47 [a] 1.07	77.72 [a] 1.53	74.34 [a] 1.43	72.89 [a] 1.58
P-value	0.3409	0.7323	0.0015	0.2691

Mean ± S.E. Values in a row with different superscript are significantly different

There were also some differences in production among the four strains with the Isa Brown and HyLine Brown tending to be more adversely affected, although these differences were not statistically significant (Table 7.2).

Table 7.2 Egg production for the four strains of bird

	Isa Brown	Hyline Brown	Hyline Grey	HiSex
T-strain	68.00 [a] 1.77	74.46 [a] 1.89	73.78 [a] 2.01	78.30 [a] 1.71
Control	72.70 [a] 1.85	78.11 [a] 1.37	74.98 [a] 1.33	78.56 [a] 1.29
P-value	0.0693	0.1211	0.6192	0.9044

Mean ± S.E. Values in a row with different superscript are significantly different

Table 7.3 Egg production for the four initial vaccination groups

	AAA	AVA	VVV	VAV
T-strain	71.12 [a] 1.92	71.94 [a] 1.99	74.13 [a] 1.70	77.34 [b] 1.93
Control	69.71 [a] 1.09	74.64 [a] 1.82	77.78 [a] 1.29	82.23 [a] 1.18
P-value	0.5238	0.3179	0.0989	0.0337

Mean ± S.E. Values in a row with different superscript are significantly different

7.4.2 Egg and Egg Shell Quality

Table 7.4 Egg shell quality of the control and challenge groups over time

		1 Wk	2 Wks	3 Wks	4 Wks	5 Wks	6 Wks	P-value
Egg Weight (g)	C	63.66 [a] ±0.42	62.43 [a] ±0.38	62.54 [a] ±0.42	62.72 [a] ±0.45	63.22 [a] ±0.39	63.51 [a] ±0.40	T <0.0001 W 0.0003 W*T 0.2173
	T	61.41 [b] ±0.36	60.38 [b] ±0.44	61.26 [b] ±0.41	62.21 [a] ±0.43	62.08 [b] ±0.38	62.71 [a] ±0.67	
Reflectivity (%)	C	45.36 [b] ±1.05	45.18 [a] ±1.03	46.13 [a] ±1.03	43.88 [a] ±0.97	44.46 [a] ±1.00	43.89 [a] ±0.96	T 0.0002 W <0.0001 W*T <0.0001
	T	49.19 [a] ±1.13	45.79 [a] ±1.08	46.60 [a] ±1.05	43.81 [a] ±1.01	44.67 [a] ±1.07	44.08 [a] ±1.01	
Breaking Strength (N)	C	36.14 [a] ±0.56	33.38 [b] ±0.57	34.40 [b] ±0.68	37.81 [a] ±0.61	37.57 [a] ±0.56	33.72 [a] ±0.59	T 0.0031 W <0.0001 W*T NS
	T	36.29 [a] ±0.69	35.36 [a] ±0.58	36.48 [a] ±0.55	38.96 [a] ±0.57	37.42 [a] ±0.57	34.43 [a] ±0.60	
Deformation (mm)	C	254.44 [a] ±5.03	257.81 [a] ±8.56	249.56 [a] ±5.77	270.06 [a] ±7.25	257.00 [a] ±5.934	239.06 [a] ±4.98	T NS W NS W*T NS
	T	254.00 [a] ±5.54	259.94 [a] ±8.15	250.63 [a] ±4.86	253.52 [b] ±4.50	255.75 [a] ±6.62	264.43 [a] ±13.75	
Shell Weight (g)	C	5.85 [a] ±0.05	5.57 [a] ±0.05	5.48 [a] ±0.07	5.93 [a] ±0.05	5.89 [a] ±0.05	5.79 [a] ±0.06	T 0.0292 W <0.0001 W*T 0.0087
	T	5.59 [b] ±0.07	5.53 [a] ±0.05	5.52 [a] ±0.06	5.88 [a] ±0.05	5.77 [a] ±0.05	5.87 [a] ±0.05	
% Shell	C	9.21 [a] ±0.07	8.92 [b] ±0.07	8.77 [b] ±0.09	9.48 [a] ±0.07	9.32 [a] ±0.06	9.13 ±0.07	T 0.0094 W <0.0001 W*T 0.0149
	T	9.12 [a] ±0.10	9.18 [a] ±0.07	9.01 [a] ±0.08	9.48 [a] ±0.08	9.32 [a] ±0.07	9.37 [a] ±0.07	
Shell Thickness (μm)	C	410.63 [a] ±2.96	398.89 [a] ±2.87	396.07 [a] ±3.52	421.73 [a] ±2.94	414.87 [a] ±2.71	409.38 [a] ±3.22	T NS W <0.0001 W*T NS
	T	408.65 [a] ±3.81	405.86 [a] ±2.95	403.10 [a] ±3.41	422.89 [a] ±3.01	413.44 [a] ±2.78	413.93 [a] ±2.79	

Means ± SEM. Means of a parameter in columns within parameters with different superscripts are significantly different. For the P values, T is challenge treatment, W is weeks of the experiment and W*T is the interaction between weeks and challenge treatment.
C is control group, T is the group challenged with T-strain IBV, NS is not statistically significant

There were statistical main effects of challenge treatment on egg weight, shell reflectivity, breaking strength, shell weight, percentage shell and yolk colour. The effects of exposure to T-strain IBV were: lower egg weight for 3 weeks postchallenge, higher reflectivity (lighter shell colour) 1 week postchallenge, and lower shell weight one week postchallenge. All measures of egg and egg shell quality changed over the six weeks of egg collections, except for deformation and albumen quality. There was a statistically significant interaction between week of the experiment and challenge treatment for shell colour, shell weight, percentage shell and yolk colour.

There were significant differences among strains for all egg quality measures (Table 7.6). In addition, there were significant differences among vaccination treatment groups for egg weight, shell colour, breaking strength, albumen quality and yolk colour (Table 7.7).

Table 7.5 Egg internal quality of the control and challenge groups over time

		1 Wk	2 Wk	3 Wks	4 Wks	5 Wks	6 Wks	P-value
Albumen Height (mm)	C	7.84 [a] ±0.10	7.60 [a] ±0.11	7.78 [a] ±0.12	7.57 [a] ±0.11	7.57 [a] ±0.11	7.64 [a] ±0.11	T NS W NS W*T NS
	T	7.61 [a] ±0.12	7.53 [a] ±0.11	7.69 [a] ±0.12	7.83 [a] ±0.12	7.71 [a] ±0.12	7.74 [a] ±0.11	
Haugh Units	C	86.66 [a] ±0.72	85.43 [a] ±0.80	86.38 [a] ±0.84	86.33 [a] ±0.78	85.03 [a] ±0.79	85.39 [a] ±0.78	T NS W NS W*T NS
	T	85.79 [a] ±0.77	85.65 [a] ±0.76	86.31 [a] ±0.77	86.84 [a] ±0.79	86.20 [a] ±0.76	86.34 [a] ±0.73	
Yolk colour	C	11.76 [b] ±0.08	11.53 [a] ±0.07	11.66 [a] ±0.10	11.49 [a] ±0.07	11.29 [a] ±0.07	11.69 [a] ±0.08	T 0.0002 W <0.0001 W*T <0.0001
	T	12.04 [a] ±0.08	10.82 [b] ±0.08	11.89 [a] ±0.08	11.24 [b] ±0.07	11.00 [b] ±0.08	11.45 [b] ±0.09	

Means ± SEM. Means of a parameter in columns within parameters with different superscripts are significantly different.
For the P values, T is challenge treatment, W is weeks of the experiment and W*T is the interaction between weeks and challenge treatment.
C is control group, T is the group challenged with T-strain IBV, NS is not statistically significant

Table 7.6 Egg quality for the four strains of bird

	Isa Brown	Hyline Brown	Hyline Grey	HiSex	P-Value
Egg Weight (g)	62.91^{a} ±0.27	62.41^{a} ±0.24	61.23^{b} ±0.21	62.83^{a} ±0.22	<0.0001
Shell Reflectivity (%)	39.58^{c} ±0.33	40.84^{b} ±0.31	64.75^{a} ±0.28	36.06^{d} ±0.26	<0.0001
Breaking Strength (N)	36.06^{b} ±0.38	35.18^{bc} ±0.31	34.38^{c} ±0.32	38.32^{a} ±0.37	<0.0001
Deformation (mm)	246.18^{a} ±3.55	252.85^{ab} ±3.37	261.55^{a} ±3.91	261.59^{a} ±5.42	0.0194
Shell Weight (g)	6.02^{a} ±0.03	5.62^{c} ±0.03	5.33^{d} ±0.03	5.92^{b} ±0.03	<0.0001
% Shell	9.59^{a} ±0.04	9.02^{c} ±0.04	8.72^{d} ±0.04	9.43^{b} ±0.04	<0.0001
Shell Thickness (μm)	433.14^{a} ±1.61	400.59^{c} ±1.50	380.71^{d} ±1.44	424.84^{b} ±1.65	<0.0001
Albumen Height (mm)	7.04^{c} ±0.07	8.07^{a} ±0.05	8.07^{a} ±0.07	7.58^{b} ±0.06	<0.0001
Haugh Units	81.72^{c} ±0.48	88.66^{a} ±0.31	88.47^{a} ±0.46	85.31^{b} ±0.43	<0.0001
Yolk Colour (Roche scale)	11.41^{b} ±0.05	11.64^{a} ±0.04	11.35^{b} ±0.05	11.54^{a} ±0.05	<0.0001

Means ± SEM. Means in rows with different superscripts are significantly different.

Table 7.7 Egg quality for the four initial vaccination groups

		AAA	AVA	VVV	VAV	P-value
Egg Weight (g)	C	63.84 [a] 0.39	61.82 [a] 0.32	63.92 [a] 0.30	62.47 [a] 0.32	T <0.0001 V <0.0001 T*V 0.1573
	T	61.62 [b] 0.31	60.94 [b] 0.29	62.66 [b] 0.37	61.45 [b] 0.32	
Reflectivity (%)	C	44.27 [a] 0.83	45.22 [a] 0.82	46.34 [a] 0.74	43.44 [b] 0.89	T 0.0002 V <0.0001 T*V <0.0001
	T	44.56 [a] 0.94	42.95 [a] 0.84	47.43 [a] 0.81	47.56 [a] 0.86	
Breaking Strength (N)	C	34.91 [a] 0.56	35.43 [b] 0.53	37.17 [a] 0.45	34.48 0.43	T 0.0031 V 0.0066 T*V 0.0022
	T	35.99 [a] 0.52	37.69 [a] 0.48	36.07 [a] 0.46	36.28 [a] 0.49	
Deformation (mm)	C	251.08 [a] 5.15	257.45 [a] 5.13	262.04 [a] 5.03	248.00 [a] 5.58	T NS V NS T*V 0.1180
	T	254.18 [a] 4.96	252.02 [a] 5.85	258.45 [a] 5.00	259.87 [a] 9.13	
Shell Weight (g)	C	5.82 [a] 0.05	5.59 [b] 0.05	5.83 [a] 0.04	5.77 [a] 0.04	T 0.0292 V NS T*V 0.0012
	T	5.66 [b] 0.05	5.75 [a] 0.04	5.71 [b] 0.05	5.67 [a] 0.05	
% Shell	C	9.12 [a] 0.06	9.05 [b] 0.07	9.14 [a] 0.05	9.24 [a] 0.05	T 0.0094 V NS T*V 0.0010
	T	9.19 [a] 0.07	9.46 [a] 0.07	9.11 [a] 0.06	9.24 [a] 0.06	
Shell Thickness (μm)	C	410.98 [a] 2.67	403.44 [a] 2.85	412.29 [a] 2.24	407.60 [a] 2.36	T NS V NS T*V <0.0001
	T	408.44 [a] 2.75	419.45 [a] 2.53	408.75 [a] 2.47	409.05 [a] 2.53	
Albumen Height (mm)	Cl	7.75 [b] 0.10	7.76 [a] 0.09	7.59 [a] 0.10	7.69 [a] 0.07	T NS V <0.0001 T*V <0.0001
	T	8.07 [a] 0.09	7.25 [b] 0.10	7.75 [a] 0.10	7.66 [a] 0.09	
Haugh Units	C	85.53 [b] 0.79	86.70 [a] 0.58	84.90 [a] 0.66	86.34 [a] 0.50	T NS V 0.0036 T*V <0.0001
	T	88.60 [a] 0.55	83.56 [b] 0.71	86.16 [a] 0.64	86.36 [a] 0.53	
Yolk Colour	C	11.57 [a] 0.06	11.47 [a] 0.07	11.79 [a] 0.06	11.45 [a] 0.07	T 0.0002 V 0.0080 T*V 0.2862
	T	11.18 [b] 0.08	11.58 [a] 0.07	11.37 [b] 0.07	11.48 [a] 0.07	

Means ± SEM. Means of a parameter in columns within parameters with different superscripts are significantly different.
C is control, T is exposed to T-strain IBV

7.4.3 Blood Analysis

There were relatively few effects of the challenge on blood measurements (Tables 7.8 to 7.17). Haematocrit increased initially in the birds exposed to T-strain IBV and plasma potassium decreased.

Table 7.8 Haematocrit treatment group means at weekly intervals.

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	28.14[a] ±0.27	29.02[a] ±0.26	30.29[a] ±0.28	28.95[a] ±0.32	28.07[a] ±0.23	28.94[a] ±0.26	28.60[a] ±0.25
T	29.10[b] ±0.27	31.02[b] ±0.34	30.82[a] ±0.34	29.51[a] ±0.35	28.14[a] ±0.23	28.77[a] ±0.38	29.31[a] ±0.29
P-value	0.0131	<0.0001	NS	NS	NS	NS	NS

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.9 Plasma sodium concentrations in mmol/L

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	150.11[b] ±0.35	147.28[a] ±0.55	147.26[a] ±0.34	150.22[a] ±0.33	148.60[a] ±0.32	150.82[a] ±0.27	150.21[a] ±0.27
T	152.35[a] ±0.34	148.29[a] ±0.40	147.18[a] ±0.41	148.48[b] ±0.43	148.16[a] ±0.35	149.18[b] ±0.31	150.45[a] ±0.25
P-value	<0.0001	NS	NS	0.0016	NS	0.0001	NS

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.10 Plasma potassium concentration (mmol/L)

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	4.84[a] ±0.05	4.82[a] ±0.05	4.77[a] ±0.04	4.72[a] ±0.05	4.72[a] ±0.04	4.91[a] ±0.04	4.86[b] ±0.03
T	4.94[a] ±0.05	4.52[b] ±0.06	4.60[b] ±0.03	4.69[a] ±0.05	4.61[a] ±0.04	4.73[b] ±0.04	4.98[a] ±0.04
P-value	NS	0.0001	0.0043	NS	NS	0.0011	0.0275

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.11 Plasma osmolarity (mmol/kg)

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	302.73[a] ±1.05	297.23[b] ±1.15	297.17[a] ±0.83	300.50[a] ±4.10	310.39[a] ±0.94	313.10[a] ±1.16	313.94[a] ±1.20
T	305.55[a] ±1.05	305.07[a] ±1.15	291.30[b] ±1.02	302.33[a] ±1.02	309.01[a] ±1.02	307.44[b] ±0.88	316.01[a] ±0.91
P-value	NS	<0.0001	<0.0001	NS	0.3252	0.0001	NS

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.12 Total plasma calcium concentration (mmol/L)

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	6.35[a] 0.33	6.45[a] 0.47	6.63[a] 0.28	7.46[a] 0.44	6.86[a] 0.43	8.56[a] 0.47	9.66[a] 0.42
T	7.28[a] 0.34	4.46[b] 0.37	7.32[a] 0.41	6.24[a] 0.49	8.04[a] 0.53	7.97[a] 0.43	7.57[b] 0.53
P-value	0.0566	0.0019	NS	NS	NS	NS	0.0036

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.13 Plasma ionised calcium

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	1.67[a] 0.02	1.54[a] 0.02	1.57[a] 0.01	1.63[a] 0.02	1.66[a] 0.02	1.76[a] 0.02	1.67[a] 0.01
T	1.68[a] 0.02	1.54[a] 0.02	1.61[a] 0.02	1.62[a] 0.02	1.62[a] 0.02	1.68[b] 0.02	1.70[a] 0.01
P-value	NS	NS	NS	NS	NS	0.0008	NS

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.14 Plasma chloride concentration

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	113.60[a] 0.95	111.40[a] 0.70	112.73[a] 0.88	112.59[a] 1.70	110.03[a] 0.66	113.14[a] 1.08	109.8[a] 1.70
T	115.90[a] 1.17	113.60[a] 0.91	110.65[a] 0.68	115.51[a] 0.71	109.31[a] 1.00	111.74[a] 0.65	112.94[a] 1.03
P-value	NS	NS	NS	NS	v	NS	NS

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.15 Plasma phosphorus concentration

	Before	1 Wk	2 Wks	3 Wks	4Wks	5 Wks	6 Wks
Control	1.72[b] 0.07	1.85[a] 0.12	1.68 [a] 0.11	1.40[a] 0.07	1.70[a] 0.08	2.00[a] 0.12	2.10[a] 0.12
T	2.08[a] 0.08	1.80[a] 0.24	1.86[a] 0.11	1.09[b] 0.06	1.73[a] 0.09	1.90[a] 0.09	2.22[a] 0.13
P-value	0.0025	NS	NS	0.0010	NS	NS	NS

Means ± SEM. Means in columns with different superscripts are significantly different.

Table 7.16 Blood analysis by vaccination group

		AAA	AVA	VVV	VAV	P-value
Na (mM)	Control	148.80 [a] ±0.26	148.88 [a] ±0.28	149.38[a] ±0.35	149.79 [a] ±0.28	0.6781
	T	148.27 [a] ±0.27	148.99 [a] ±0.30	149.67 [a] ±0.30	149.74 [a] ±0.33	
K (mM)	Control	4.77[a] ±0.04	4.81[a] ±0.03	4.75[a] ±0.04	4.88[a] ±0.03	0.6993
	T	4.72[a] ±0.03	4.76[b] ±0.04	4.70[a] ±0.04	4.73[a] ±0.04	
Ca^{++} (mM)	Control	1.65[a] ±0.01	1.66 [a] ±0.01	1.62 [a] ±0.02	1.64 [a] ±0.01	0.9110
	T	1.64 [a] ±0.01	1.66 [a] ±0.01	1.60 [a] ±0.02	1.64 [a] ±0.02	
Hct (%)	Control	28.61 [a] ±0.20	28.52[b] ±0.22	29.83 [a] ±0.22	28.50[b] ±0.20	0.2746
	T	28.78 [a] ±0.23	29.96[a] ±0.28	29.77 [a] ±0.26	29.31[a] ±0.27	
Osm (mmol/kg)	Control	304.31 [a] ±0.88	304.56 [a] ±0.86	303.39 [a] ±2.52	307.66 [a] ±1.15	0.6245
	T	304.32 [a] ±0.96	305.56 [a] ±0.90	304.81 [a] ±1.12	306.41 [a] ±1.13	
Cl (mM)	Control	111.47 [a] ±0.60	111.00 [a] ±0.52	113.34 [a] ±1.09	111.83 [a] ±1.14	0.4595
	T	113.31 [a] ±0.76	110.44 [a] ±0.64	114.25 [a] ±0.62	113.13 [a] ±0.75	
Total Ca (mM)	Control	7.49 [a] ±0.32	6.50 [a] ±0.40	6.95 [a] ±0.37	7.52 [a] ±0.25	0.7210
	T	7.05 [a] ±0.34	7.08 [a] ±0.49	7.24 [a] ±0.39	7.73 [a] ±0.41	
P (mM)	Control	1.80 [a] ±0.07	1.71 [a] ±0.11	1.70 [a] ±0.08	1.89 [a] ±0.07	0.8276
	T	1.82 [a] ±0.12	1.63 [a] ±0.10	1.84 [a] ±0.12	1.95 [a] ±0.10	

Means ± SEM. Means in columns within parameters, with different superscripts are significantly different.

Table 7.17 Blood analysis by strain of bird.

		Isa Brown	Hyline Brown	Hyline Grey	HiSex	P-value
Na (mM)	Cl	148.96[a] ±0.32	149.52[a] ±0.28	148.86[a] ±0.30	149.49[a] ±0.28	0.1726
	T	149.81[a] ±0.32	149.08[a] ±0.32	148.24[a] ±0.29	149.45[a] ±0.28	
K (mM)	C	4.81[a] ±0.03	4.85[a] ±0.03	4.75[a] ±0.04	4.80[a] ±0.31	0.5883
	T	4.78[a] ±0.03	4.70[b] ±0.04	4.66[a] ±0.04	4.76[a] ±0.04	
Ca^{++} (mM)	Cl	1.63[a] ±0.02	1.65[a] ±0.02	1.63[a] ±0.02	1.65[a] ±0.01	0.1758
	T	1.66[a] ±0.02	1.63[a] ±0.02	1.64[a] ±0.01	1.61[a] ±0.01	
Hct (%)	C	28.63[a] ±0.21	29.26[b] ±0.19	28.61[a] ±0.24	28.91[b] ±0.20	0.0795
	T	29.02[a] ±0.21	31.07[a] ±0.33	28.02[a] ±0.19	30.02[a] ±0.23	
Osm (mmol/kg)	C	304.59[a] ±0.89	303.69[a] ±2.35	304.93[a] ±1.19	306.80[a] ±1.01	0.5388
	T	305.76[a] ±1.16	305.39[a] ±0.95	304.28[a] ±0.94	305.52[a] ±1.04	
Cl (mM)	C	112.67[a] ±0.75	112.28[a] ±1.00	109.64[a] ±1.05	112.93[a] ±0.66	0.8003
	T	113.41[a] ±0.66	112.81[a] ±0.69	111.61[a] ±0.82	113.37[a] ±0.62	

Means ± SEM. Means in columns within parameters, with different superscripts are significantly different.
C is control, T is the group exposed to T-strain IBV

7.4.4 Kidney Weights

There were no effects of any of the experimental variables on kidney weight in this experiment. When the differences in kidney weights are compared for the three challenge trials (C1 at 25, C2 at 41 and C3 at 62 weeks), the only age at which T-strain IBV had a significant effect on kidney weight was at 25 weeks (Table 7.20).

Table 7.18 Kidney weights for the experimental groups

	Control	T-strain	P-value
R/L ratio	0.97 ±0.01	1.01 ±0.01	NS
Kidney weight as % body Wt.	0.65[a] ±0.02	0.65[a] ±0.04	NS

Table of means ± SEM. R/L is right to left ratio

Table 7.19 Kidney weights for the four strains of bird

		Isa Brown	Hyline Brown	Hyline Grey	HiSex	P-value
R/L ratio	Control	0.96[a] ±0.02	0.99[a] ±0.02	0.97[a] ±0.02	0.98[a] ±0.02	NS
	T-strain	0.99[a] ±0.03	1.04[a] ±0.02	1.01[a] ±0.03	1.01[a] ±0.02	NS
Kidney weight as % body Wt.	Control	0.66[a] ±0.02	0.63[a] ±0.03	0.66[a] ±0.04	0.66[a] ±0.04	NS
	T-strain	0.61[a] ±0.03	0.65[a] ±0.03	0.79[a] ±0.14	0.56[a] ±0.04	NS

Table of means ± SEM. R/L is right to left ratio

Table 7.20 Total kidney weight as a percentage of body weight

	Control	T-strain	P-value
C1	0.57^{b} ±0.02	0.67^{a} ±0.04	0.0105
C2	0.64^{a} ±0.01	0.65^{a} ±0.02	0.5277
C3	0.65^{a} ±0.02	0.65^{a} ±0.04	0.9933

Table of means ± SEM. Means in rows with different superscripts are significantly different.
C1= challenge at 25 wks, C2= challenge at 41 wks, C3= challenge at 62 wks

7.4.5 Manure Moisture

Manure moisture was significantly increased by exposure to T-strain IBV. This occurred at all 4 weeks of excreta collections (Table 7.21), all strains of bird (Table 7.22) and all initial vaccination treatment groups (Table 7.23).

Table 7.21 Percent manure moisture at weekly intervals after challenge.

	1 Week	2 Weeks	3 Weeks	4 Weeks
Control	67.57^{b} ±0.59	63.85^{b} ±0.75	63.40^{b} ±0.83	62.55^{a} ±0.62
T-strain	72.09^{a} ±1.23	68.00^{a} ±0.62	66.66^{a} ±1.06	67.95^{b} ±0.69
P-value	0.0013	<0.0001	0.0170	<0.0001

Table of means ± SEM. Different superscripts in columns indicate significant differences.

Table 7.22 Percent manure moisture of the four strains of bird.

	Isa Brown	Hyline Brown	Hyline Grey	Hisex
Control	65.10^{a} ±0.75	65.02^{b} ±0.73	64.54^{b} ±0.78	62.69^{b} ±0.71
T-strain	66.95^{a} ±0.70	70.49^{a} ±1.03	71.20^{a} ±1.11	66.07^{a} ±0.81
P-value	0.0758	<0.0001	<0.0001	0.0023

Table of means ± SEM. Different superscripts in columns indicate significant differences.

Table 7.23 Percent manure moisture of the four initial vaccination groups

	AAA	AVA	VVV	VAV
Control	64.61[b] ±0.90	64.49[b] ±0.52	64.93[b] ±0.86	63.34[b] ±0.67
T-strain	67.26[a] ±0.72	69.28[a] ±0.85	70.33[a] ±1.17	67.85[a] ±1.06
P-value	0.0230	<0.0001	0.0003	0.0005

Table of means ± SEM. Different superscripts in columns indicate significant differences.

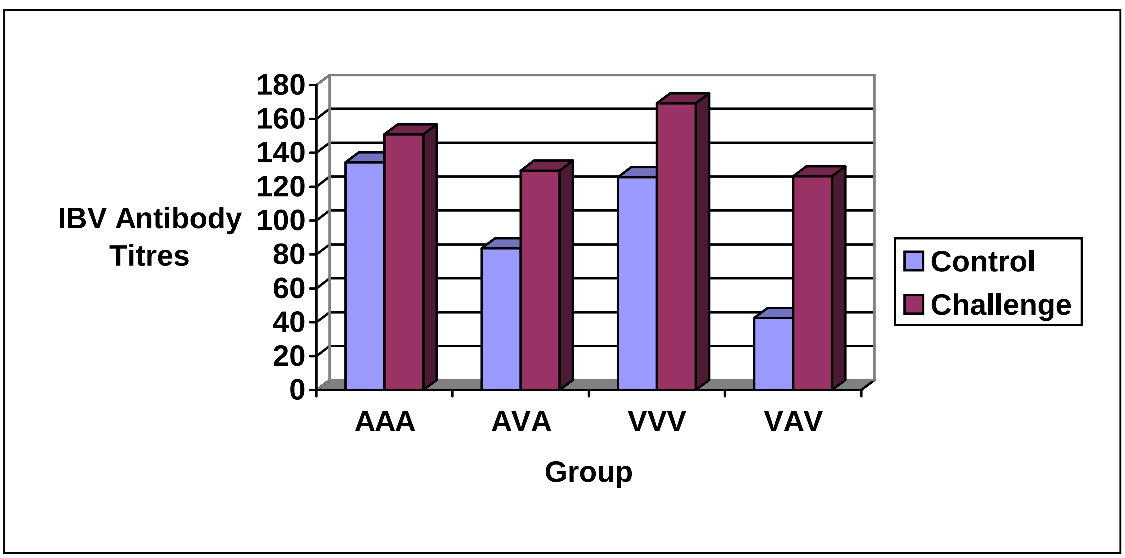
7.4.6 Histology

The right caudal division of the kidneys from the C3 (challenge at 62 wks) Isa Brown birds has so far been examined histologically. There were histological lesions in two of the eight infected kidneys examined. One of these kidneys had extensive areas of fairly light lymphocyte infiltration and the other had some oedema of the capsules of the reptilian-type glomeruli, these kidneys had no necrosis or other observed histopathologies. Kidneys of birds from the control group were unaffected.

7.4.7 IBV Antibody Titres

There was no significant effect of challenge with T-strain IBV on the IBV antibody titres 3 weeks following challenge (Figure 7.1) in Isa Brown birds. However, there was a significant effect of original vaccination group and a significant interaction between the effects of challenge and original vaccination group. AAA indicates the group that received A3 vaccine at day-old, 4 weeks and 14 weeks of age; AVA indicates A3 at day-old, VicS at 4 weeks and A3 at 14 weeks; VVV is VicS at day-old, 4 and 14 weeks; VAV is VicS at day-old, A3 at 4 weeks and VicS at 14 weeks. Titres before and 3 weeks after challenge, combined, were highest in the VVV and AAA groups, lowest in the VAV group, with the AVA group intermediate. There was considerable variation in titre levels among individual birds. The overall pattern may be clearer when the blood samples have been analysed from all the strains of bird.

Figure 7.2 IBV antibody titres in different vaccination groups of Isa Brown birds in control and challenge groups 3 weeks after challenge with T-strain virus



7.5 Discussion and Conclusions

The results of this study indicate that birds that had not been vaccinated since the third vaccination at 14 weeks of age were negatively affected in a range of ways when they were challenged with T-strain IBV at 62 weeks of age. Production dropped for at least 6 weeks postchallenge and there were negative effects on egg and egg shell quality. Egg weight was reduced as was shell weight and shell colour was lighter. Effects on albumen quality had been expected but were not found in this study.

There were relatively few effects of challenge with T-strain on blood measurements although haematocrit increased initially and there was an effect on plasma potassium. Kidney weight was not affected but there were some histopathological changes in the kidneys of some of the challenged birds. The fact that manure moisture increased in the challenged birds suggests that there are negative effects on either the kidneys or the digestive tract.

The IBV antibody titres, measured by TropBio ELISA showed a general increase in titre following challenge. However, the results were less clear-cut than for the challenges conducted at 25 and 41 weeks, owing to more variable titres at commencement of the experiment.

8. Trial 2: Effects of vaccine strain in providing protection against challenge with T-strain infectious bronchitis – kidney function experiments

8.1 Introduction

This study used birds from Trial 2 which had not been utilised in the challenge experiments conducted at 25, 41 and 62 weeks of age. Conventional methods of determining kidney function were applied at 70 weeks of age to vaccinated birds that had (or had not) been exposed to T-strain infectious bronchitis virus at the end of lay, after not having been vaccinated since they were 14 weeks of age. The kidney damage caused by T-strain infection is reported to cause excessive water, sodium and potassium loss from susceptible birds (Heath, 1970). Therefore, it would be expected that differences in the kidney function parameters would be observed in challenged birds unless they were protected from the actions of the virus to some degree.

8.2 Objectives

The aim of this study was to evaluate the degree of protection afforded by early vaccination against the nephropathogenic effects of challenge with T-strain infectious bronchitis virus.

8.3 Methodology

Forty commercial laying pullets were vaccinated with a commercial infectious bronchitis vaccine at day old, 4 weeks and 14 weeks, by coarse spray. The birds were initially raised on the floor and at 13 weeks of age the pullets were transferred to individual cages in large isolation sheds. At 70 weeks of age the birds were divided into 2 treatment groups, one group (T-strain) were exposed intraocularly to T-strain infectious bronchitis virus, the other group did not receive the virus (Control).

At 5, 10, 14 and 19 days post-exposure, 5 birds were removed from each of the two groups for kidney function experiments. Each bird was anaesthetised by intramuscular injection of DIAL (5,5 diallyl-barbituric acid) and body weight measured. Once the anaesthetic had taken effect, cannulae were placed in the left brachial vein and one of the carotid arteries. An initial blood sample was then taken. A solution of mannitol, inulin and para-aminohippuric acid (PAH) was infused via the brachial cannula. The lower gut was emptied and tied off to prevent faecal contamination of the urine samples. The birds were allowed 40-60 minutes for their system to equilibrate after the commencement of infusion. Urine collection was carried out over thirty minutes with a blood sample taken at the time mid-point. This process was then repeated for a second urine collection period. Upon completion of the second clearance interval the bird was euthanased with an intravenous lethal dose of barbiturate.

On day 14 post-exposure the kidneys of each bird were removed, weighed and sections fixed for histological examination, in 10% formalin, and the remnants frozen for virological analysis.

Blood samples were sub-sampled and haematocrit value measured, the remainder was centrifuged at 3000 rpm for 10 mins and the plasma supernate removed and frozen for later analysis. 1.5 and 1 mL sub samples of the urine collections were taken, the 1.5mL sample was centrifuged and frozen for ion and plasma osmolarity analysis and the 1mL sample was diluted with 1mL of 0.5M lithium hydroxide for the inulin and PAH assays. After the analyses were carried out the glomerular filtration rate, effective renal plasma flow, effective renal blood flow, ion tubular load, excretion rate, fractional excretion, fractional reabsorption, osmolar clearance and freewater clearance were calculated. A determination of analysis of variance was then carried out on the results.

Histological samples, the caudal section of the right kidney of each bird, were prepared and stained with haematoxylin/Eosin or Alcian Blue and examined for pathological changes.

Virus isolation in chicken embryos, as per the method of Cunningham (1973), was carried out on the kidney tissue retrieved at 14 days post-exposure that was in excess of the requirements of the histological analysis. The homogenised kidney samples were passaged four times through eggs to reach a determination of negative or positive for infectious bronchitis virus.

8.4 Detailed Results

8.4.1 Kidney Function Analysis

Glomerular filtration rate (GFR) was found to be significantly higher in the T-strain birds at 5 days post-exposure to infection when compared to the control birds at the same time. Table 8.1 provides the mean values for the both groups of five birds on each day of analysis. There were no significant differences between the T-strain and Control groups on any of the other analysis days.

Table 8.1 Glomerular filtration rate (mL/min/kg) at intervals from the day of infection.

Treatment Group	5 days	10 days	14 days	19 days
Control	1.27^{a} ±0.074	2.20^{a} ±0.220	2.46^{a} ±0.449	1.605^{a} ±0.199
T-strain	2.46^{b} ±0.287	2.32^{a} ±0.180	2.78^{a} ±0.366	1.67^{a} ±0.140
P-Value	0.003	0.685	0.599	0.799

Values are means ± standard error. Values in columns with different superscripts are significantly different (P<0.05).

There was no significant effect of treatment group or time after exposure to the virus on effective renal plasma flow (ERPF) or effective renal blood flow (ERBF). Sodium and potassium tubular load were significantly increased in the T-strain birds at 5 days after exposure (Table 8.2).

Table 8.2 Sodium and potassium tubular load (T.L. mmol/min) at 5 days post-exposure.

	Control	T-Strain	P- Value
Sodium T.L.	0.474 ±0.046	0.812 ±0.046	0.0013
Potassium T.L.	0.008 ±0.001	0.014 ±0.001	0.0002

Values are means ± standard error.

Sodium and potassium fractional excretion (F.E.) was not significantly different between the control and T-strain birds. However, there was a trend toward increased excretion of these ions by the T-strain birds at 5 days post-infection. Due to their method of calculation this is the converse of the results for sodium and potassium fractional reabsorption, a slightly higher value for the T-strain birds at 5 days after infection, but not statistically significant. Neither the treatment group nor the time after infection had any significant effect on the renal chloride excretion/reabsorption. There were no significant effects on osmolarity clearance, freewater clearance, haematocrit value (Hct), urine flow rate or plasma osmolarity. However, there is a trend for the T-strain birds to have lower haematocrit (Table 8.3).

Table 8.3 Plasma osmolarity of treatment groups across the duration of the experiment.

	Control	T-strain	P-Value
Hct (%)	28.25 ±0.56	26.88 ±0.57	0.097
Plasma Osmolarity (mmol/kg)	323.95 ±4.01	331.11 ±4.14	0.221

Values are means ± standard error. $P< 0.05$ indicates statistical significance.

8.4.2 Kidney weights

There were no significant effects of exposure to T-strain infectious bronchitis on the kidney weights. The calculations carried out were for right and left kidneys each as a percentage of body weight, the total weight of the kidneys as a percent of body weight and the ratio of heavy kidney to light as an indicator of asymmetry. There were no effects of treatment on any of these parameters (Table 8.4).

Table 8.4 Kidney weight group means.

	Right kidney as % Bwt	Left kidney as % Bwt	Total kidney as % Bwt	Heavy/Light kidney ratio
Control	0.364 ±0.019	0.348 ±0.018	0.712 ±0.038	1.045 ±0.018
T-strain	0.380 ±0.014	0.379 ±0.016	0.759 ±0.026	1.075 ±0.012
P-value	0.521	0.239	0.339	0.210

Values are means ± standard error. Bwt = Body weight.

8.4.3 Histological analysis

There were no pathological changes observed in the kidneys of the control birds. One of the five birds exposed to T-strain showed one small area of mild lymphocyte infiltration. The other 4 kidneys showed no histopathological lesions.

8.4.4 Virus Isolation

Infectious bronchitis virus was successfully isolated from the kidneys of all five challenged birds from whom isolation was attempted. After four passages of kidney homogenate samples in embryonated eggs there were no effects visible in the eggs injected with Control group sample.

8.5 Discussion and Conclusions

The statistically significant ($P<0.05$) difference observed in the glomerular filtration rate (GFR) at day five post-exposure of one group to T-strain may be due to a combination of the control birds being slightly below the mean on this day and the T-birds being above it. GFR is known to decrease in dehydrated birds (Roberts and Dantzler, 1989) so the hydration state of the control birds may account for the differences. However, there were no effects of treatment group on the plasma osmolarity and haematocrit value, indicating no significant differences between the hydration states of the two groups. There was a trend to a higher haematocrit in the control group, which probably accounts for the slightly lowered control group GFR on day 5, but it cannot account for all of the significant increase in GFR for the T-strain birds at this time. Apart from this increase in GFR at day 5, there were no differences between the functioning of the kidneys of exposed birds and those of the unexposed control birds.

Tubular ion load is calculated from the product of GFR and plasma ion concentration. Therefore the sodium and potassium tubular loads are significantly higher for the T-strain infected treatment group at the 5-day collection due to the increased GFR at this time. Despite this increased presence of ions to be filtered in the glomeruli the total excretion rate, fractional excretion and fractional reabsorption were not altered significantly from the control. Hence the kidneys of the IB exposed birds appeared to be compensating for the increased GFR, resulting in no excessive loss of these two ions.

The slight trend toward a lower haematocrit in the T-strain birds is not significant, however it is consistent with previous work on T-strain (Afanador and Roberts, 1994). It is suggested that birds exposed to T-strain infectious bronchitis are losing excess water due to inefficient virus-damaged kidneys, and therefore drink more water, over compensate and this results in a larger percentage plasma by volume (and lower haematocrit value). The results of this trial showed a slight trend toward a similar response, but were not statistically significant. Therefore if this was what was indeed occurring, it was of a mild and short-lived nature. The vaccinated challenged birds (T-strain birds) in the current trial had no significant changes in the osmolar clearance, freewater clearance and urine flow rate when compared to the control birds. This indicates that the infected birds are not losing statistically significant excessive amounts of water through the kidneys, and consequently are protected by the early vaccinations that they received.

Glomerular filtration rate can increase with expansion of the bird’s extracellular fluid (Roberts, 1992). The trend to a decreased haematocrit value in the T-strain treatment group might indicate that they are drinking more, as mentioned above, and consequently the extracellular fluid may have increased, increasing the GFR. If this is the case it is not occurring to a degree that has significantly increased the urine flow rate. Therefore the impact of any increased water consumption on increased GFR is slight and transient in these vaccinated birds.

The presence of one area of mild lymphocyte infiltration in the right caudal kidney section of only one of the five T-strain birds also suggests a fairly high level of protection against T-strain is still afforded by the three early vaccinations. In addition to the lack of histopathological changes there is the evidence of the kidney weights and asymmetrical ratios being not statistically different.

The batch of virus used to challenge the birds in this trial had previously caused macroscopic kidney lesions and ten percent mortality in cockerels naïve to infectious bronchitis virus (unpublished data). The isolation of virus from the kidney tissue of the T-strain group but not the Control group indicates that the experimental group of birds was exposed to infection with a nephropathogenically active challenge virus.

The birds in this trial had been vaccinated three times before the point of lay and then not vaccinated again until, at 70 weeks of age, half of the group were exposed to T-strain infectious bronchitis. The well-documented nephropathogenic nature of T-strain IBV should have resulted in significant declines in the traditional measurements of kidney function. The apparent lack of any real statistically significant detrimental effects of the T-strain on these birds enables us to conclude that their kidneys are still adequately protected from their early vaccination schedule. From this it would appear that revaccination during lay is unnecessary if the birds are vaccinated correctly during the rearing phase. Indeed revaccination may in itself have deleterious effects on egg and eggshell quality in the commercial laying hen (Sulaiman *et al*, 2002).

9. Comparison of serological methods

9.1 Introduction

It is essential that the industry has tools for surveillance of layer flocks for effects of infectious bronchitis virus. The level of protection afforded by vaccination is usually measured in the form of IBV antibody titres. The most common method of doing this is to use commercially available IBV antibody ELISA kits. There are currently three available: KPL ProFlok; IDEXX and TropBio. The TropBio kit is the only one that was developed specifically for Australian conditions. However, the other two kits are also in common commercial use, especially IDEXX. Other methods of measuring IBV antibody titres include serum neutralisation, agar gel precipitation and haemagglutination inhibition. These tests are not always readily available commercially and are more time-consuming and expensive than ELISA.

9.2 Objectives

The aim of this chapter is to provide some preliminary data on comparisons among the different methods of measuring IBV antibody titres.

9.3 Methodology

IBV antibody titres were measured using an IDEXX IBV antibody ELISA kit and were conducted by Birling Avian Laboratories. Agar gel precipitation (AGP) tests which give a positive/negative result were conducted after the method of Woernle (1966) (Chubb and Cumming, 1971). Serum neutralisation (SN) tests were conducted after the method of Fabricant (1951). Serum neutralisation tests were conducted to identify positive or negative only. It is possible to measure antibody titres using SN but this requires 25 eggs per sample, rather than the 5 required for just a positive/negative result.

9.4 Detailed Results

Figures 9.1 and 9.2 show the results of IBV antibody titres measured by IDEXX ELISA. The patterns are very similar for the birds that were revaccinated regularly during lay and those that were not. This raises issues of interpretation of IBV antibody titres measured by ELISA.

Table 9.1 shows the results from IDEXX ELISA along with APG and SN for 4 of the sets of blood samples that were taken during Trial 1 (and shown in Figures 9.1 and 9.2). The same 70 birds were bled at each age.

Table 9.2 shows the same data sorted in order of IDEXX IBV antibody titre.

Figure 9.3 is a comparison of IBV antibody titres measured by two different ELISA kits, IDEXX and TropBio at the same laboratory at the University of Melbourne. While there is a linear relationship between the two sets of values, there is not always one-on-one matching.

Figure 9.1 IDEXX IBV antibodies of birds revaccinated regularly during lay (Trial 1)

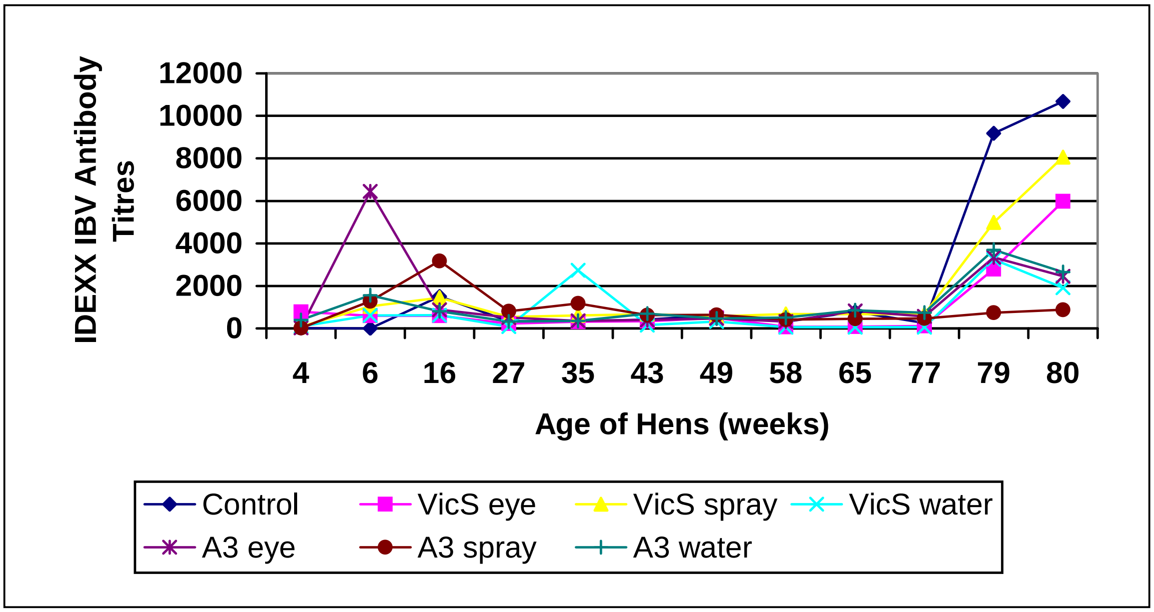


Figure 9.2 IDEXX IBV antibodies of birds not revaccinated regularly during lay (Trial 1)

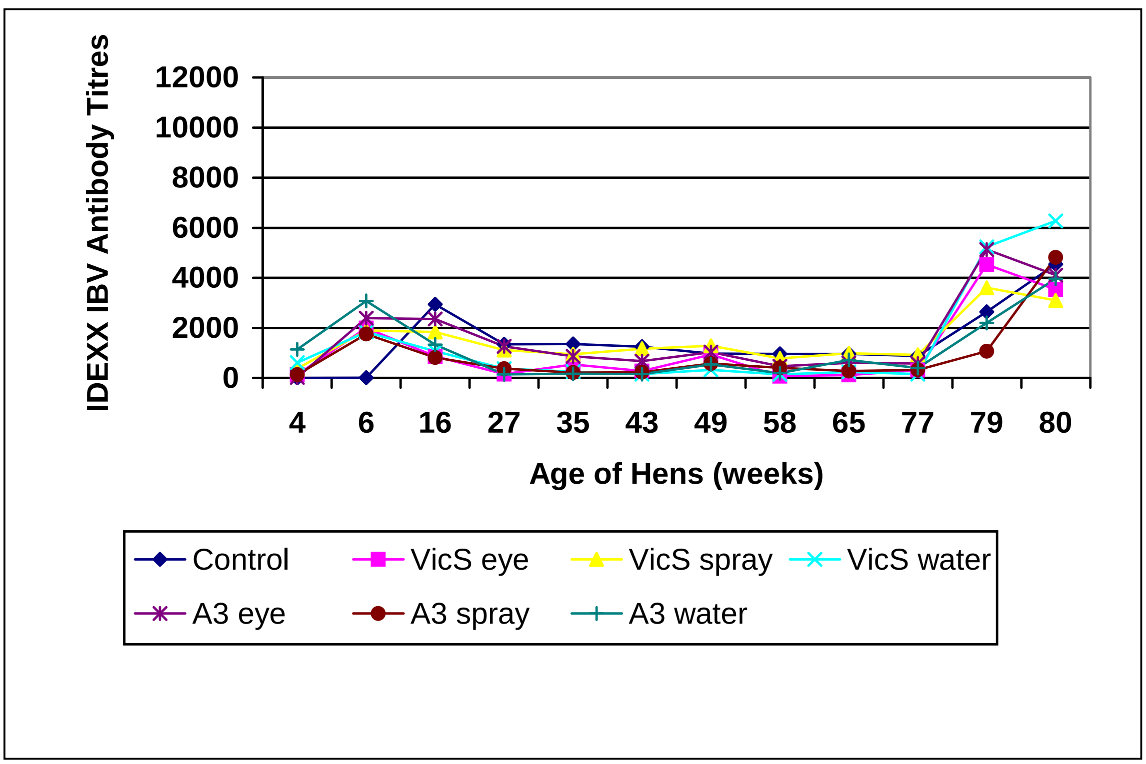
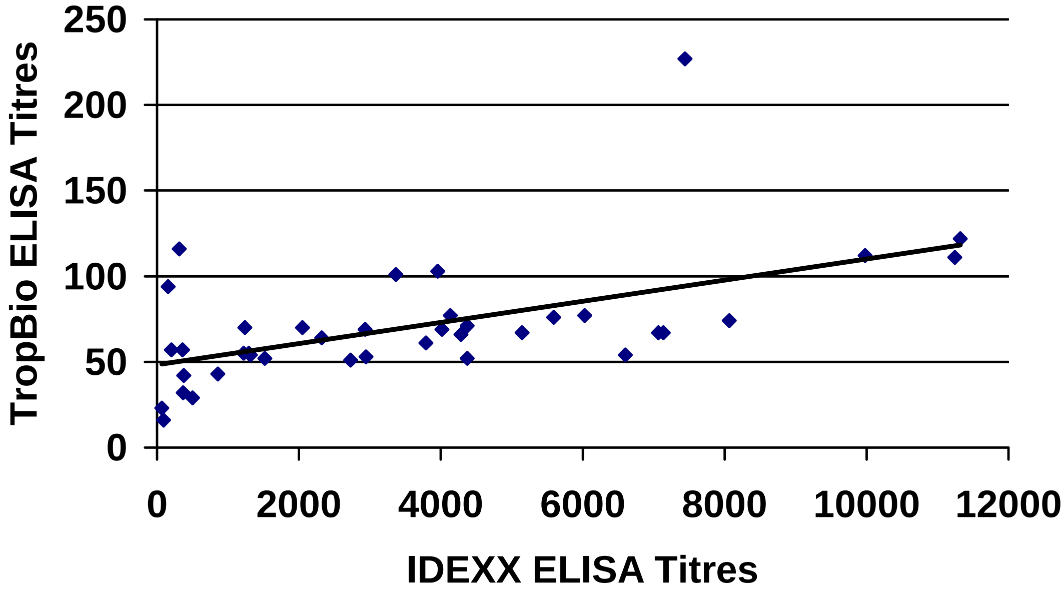


Figure 9.3 Comparison of IDEXX and TropBio ELISA kits



The percentage of samples at the 4 different ages which were positive by IDEXX ELISA, AGP or SN tests are shown in Table 9.3. The ELISA measured a percentage of positive samples at 6 and 16 weeks, as compared with the AGP and SN tests. The comparison of IBV antibody titres measured by IDEXX IBV antibody ELISA and the positive/negative results obtained from AGP and SN tests is shown in Figure 9.4. The ELISA antibody titres have been grouped to allow for the calculation of percentage positive results for AGP and SN. The sample sizes for the groupings are: 0-100, 33 samples; 101-200, 34 samples; 201-500, 48 samples; 501-1000, 38 samples; 1001-2000, 35 samples; 2001-4000, 30 samples; 4001-6000, 21 samples; 6001-8000, 19 samples; 8001-15000 12 samples. There is a general increase in the percentage of samples testing positive for AGP and SN, as the ELISA antibody titre increases. The mean IBV antibody ELISA titre of the birds, just prior to exposure to T-strain IBV was 439 (see also Table 9.3). This titre level correlated with a high level of protection of the birds as there were no significant effects on production or kidney histopathology.

Table 9.1 Comparison with ELISA IBV antibody titres (IDEXX) with SN and AGP

	Treatment	Re-vac	Week 6			Week 16			Week 58			Week 80		
		Y/N	IDEXX	SN	AGP	IDEXX	SN	AGP	IDEXX	SN	AGP	IDEXX	SN	AGP
1	CONTROL	N	1	Neg	Neg	4033	Neg	Neg	219	Neg	?	541	Neg	Neg
2	CONTROL	Y	15	Neg	Neg	1368	Neg	Neg	321	Pos/Neg	?	11315	Neg	Pos
3	CONTROL	N	1	Neg	Neg	360	Neg	Neg	219	Neg	Pos	7946	Pos	Pos
4	CONTROL	Y	1	Neg	Neg	1818	Neg	Neg	182	Neg	Pos	10364	Pos	-
5	CONTROL	N	1	Neg	Neg	6434	Pos	Pos	1584	Pos	Pos	2134	Pos	Neg
6	CONTROL	N	39	Neg	Neg	3247	Neg	Neg	207	Neg	Neg	298	Pos	Neg
7	CONTROL	Y	1	Neg	Neg	2883	Neg	Neg	225	Neg	Neg	10082	Pos	Pos
8	CONTROL	Y	1	Neg	Neg	964	Neg	Neg	718	Neg	Neg	10647	Pos	Pos
9	CONTROL	Y	1	Neg	Neg	444	Neg	Neg	86	Neg	Neg	10991	Pos	Pos
10	CONTROL	N	25	Neg	Pos?	612	Neg	Neg	2582	Pos	Neg	11763	Pos	Pos
11	VICSEYE	N	1882	Neg	Neg	262	Neg	Neg	103	Neg	?	959	Pos/Neg	?
12	VICSEYE	Y	1180	Neg	Neg?	242	Neg	Neg	97	Pos/Neg	?	8540	Pos	Pos
13	VICSEYE	Y	383	Neg	Neg	172	Neg	Neg	150	Neg	Neg	7463	Pos	Pos
14	VICSEYE	N	673	Neg	Neg	192	Neg	Neg	250	Neg	Neg	.	Neg	?
15	VICSEYE	Y	132	Pos/Neg	Neg	711	Neg	Neg	150	Neg	Pos	11196	Pos	Pos
16	VICSEYE	N	5825	Neg	Neg	95	Neg	Neg	24	Neg	?	401	Neg	Neg
17	VICSEYE	Y	405	Neg	Neg	105	Neg	Pos	9	Neg	?	1387	Pos	Pos
18	VICSEYE	Y	851	Pos	Neg	1747	Neg	Neg?	24	Neg	?	6518	Pos	Pos
19	VICSEYE	N	1076	Neg	Neg	3157	Neg	Pos	1	Neg	Neg	8693	Pos/Neg	Pos
20	VICSEYE	N	510	Neg	Neg	592	Neg	Pos	138	Neg	Pos	4056	Neg	Pos

21	VICSSPY	N	4530	Pos	Neg	5529	Pos	Pos	156	Pos	?	229	Pos	Neg
22	VICSSPY	N	973	Neg	Neg	444	Pos	Pos	109	Neg	Neg	5757	Pos	Neg
23	VICSSPY	Y	457	Neg	?	67	Neg	Neg	156	Neg	Neg	855	Pos	Pos
24	VICSSPY	Y	142	Neg	?	477	Pos/Ne	Pos	114	Pos/Neg	Neg	5717	Pos	Pos

Table 9.1 (cont)

	Treatment	Re-vac	Week 6			Week 16			Week 58			Week 80		
		Y/N	IDEXX	SN	AGP	IDEXX	SN	AGP	IDEXX	SN	AGP	IDEXX	SN	AGP
25	VICSSPY	N	906	Neg	Neg	1640	Pos	Pos	2620	Pos	Pos	6100	Pos	Pos
26	VICSSPY	N	1919	Neg	Neg	1046	Pos	Pos	138	Neg	Neg	-	Pos	Pos
27	VICSSPY	Y	1318	Neg	Neg	2542	Pos	Neg	1454	Pos	Neg	14253	Pos	Pos
28	VICSSPY	Y	510	Neg	Neg	3126	Neg	Pos	976	Neg	Pos	10971	Pos	Pos
29	VICSSPY	Y	2745	Neg	Neg	999	Neg	Pos	668	Pos	Pos	8498	Neg	Pos
30	VICSSPY	N	1133	Neg	Neg	552	Neg	Pos	219	Pos	Pos	324	Pos	Neg
31	VICSWTR	Y	300	Neg	Neg	142	Neg	Neg	24	Neg	Pos?	50	Neg	?
32	VICSWTR	N	985	Neg	Neg	54	Neg	Neg	34	Neg	?	6206	Pos	Pos
33	VICSWTR	Y	2025	Neg	Neg	302	Neg	Neg	366	Neg	?	6404	Pos	Pos
34	VICSWTR	Y	152	Neg	Neg	1730	Pos	Neg	86	Pos	Neg	3319	Pos	-
35	VICSWTR	N	4670	Neg	Neg	4482	Pos/Neg	Neg	431	Pos/Neg	Neg	5889	Pos	?
36	VICSWTR	Y	427	Neg	Neg	313	Neg	Neg	69	Pos/Neg	Neg	3085	Pos/Neg	Pos
37	VICSWTR	N	2461	Neg	Neg	319	Neg	Neg	45	Neg	Neg	4532	Pos	Pos
38	VICSWTR	N	7	Neg	Neg	114	Neg	Neg	50	Neg	Neg	8844	Pos	Pos
39	VICSWTR	N	1041	Neg	Neg,Pos?	360	Neg	Neg	63	Neg	Neg	.	Neg	Pos
40	VICSWTR	Y	160	Neg	Neg	635	Pos	Neg	74	Neg	Neg	800	Pos	Pos?
41	A3SPY	N	1481	Pos	?,Neg	1522	Pos/Neg	Pos	1199	Pos	Neg	2663	Pos	Pos
42	A3SPY	N	1399	Pos/Neg	?,?	252	Pos	Pos	182	Pos	Neg	6092	Pos	Pos
43	A3SPY	Y	1774	Pos	Neg	646	Pos	Pos	114	Pos/Neg	Pos	1738	Pos	Pos
44	A3SPY	N	5108	Pos	Pos	349	Pos	Neg	138	Pos	?	8145	Pos	Pos
45	A3SPY	Y	1	Neg	Pos	1039	Pos	Pos	200	Neg	Pos	330	Pos	Neg
46	A3SPY	Y	2978	Pos	Pos	3208	Pos	Pos	1090	Neg	Pos	1777	Pos	Pos
47	A3SPY	N	739	Pos	Neg	835	Pos	Pos	114	Neg	Neg	2388	Pos	Pos
48	A3SPY	Y	997	Pos	Neg/Pos	3308	Pos	Pos	182	Neg	Pos	254	Pos/Neg	Neg

Table 9.1 (cont)

	Treatment	Re-vac	Week 6			Week 16			Week 58			Week 80		
		Y/N	IDEXX	SN	AGP	IDEXX	SN	AGP	IDEXX	SN	AGP	IDEXX	SN	AGP
49	A3SPY	Y	608	Pos	Neg	7689	Pos	Pos	466	Pos/Neg	Pos	279	Neg	Pos
50	A3SPY	N	67	Pos	Neg	1114	Pos	Neg	614	Pos	?	.	Pos	Neg?
51	A3WTR	Y	5007	Pos	?,neg	1694	Pos	Neg	412	Pos	Pos	3404	Pos	Pos
52	A3WTR	N	3388	Pos	??	172	Pos	Neg	103	Neg	Neg	2746	Pos	Pos
53	A3WTR	Y	4363	Pos	Neg,?	9300	Pos	Pos	1469	Pos	Neg	1372	Pos	?
54	A3WTR	Y	300	Neg	Neg	360	Neg	Pos	176	Pos	Pos	1983	Pos	Pos
55	A3WTR	N	985	Neg	Pos,Neg	3165	Pos	Pos	288	Pos	Pos	.	Pos	Pos
56	A3WTR	N	1786	Pos	Pos,Neg?	2889	Pos	Neg	334	Neg	Pos	3740	Pos	Pos
57	A3WTR	Y	416	Pos	Neg	334	Pos	Neg	69	Neg	Neg	4300	Pos	Pos
58	A3WTR	N	8123	Neg	Pos	48	Neg	Neg	207	Neg	Neg	138	Pos	?
59	A3WTR	Y	490	Pos	?	823	Pos/Neg	Pos	156	Pos	Pos	904	Pos	Pos
60	A3WTR	N	1121	Pos	Neg	366	Pos	Neg	103	Pos/Neg	Pos	9232	Neg	Pos
61	A3EYE	Y	5408	Neg	-	1067	Pos	Neg	150	Pos/Neg	Neg	113	Pos	?
62	A3EYE	N	510	Neg	Neg	673	Neg	Neg	244	Pos/Neg	Pos	2283	Pos	Pos
63	A3EYE	Y	4696	Pos	Neg	796	Pos	Pos	244	Pos	Pos	.	Pos	Neg
64	A3EYE	N	343	Pos	Neg	3847	Neg	Pos	828	Pos	Pos	3054	Pos	-
65	A3EYE	N	4903	Pos	Pos	3821	Pos	Pos	628	Pos/Neg	Neg	1008	Pos	Neg
66	A3EYE	Y	7400	Pos	Pos	138	Neg	Neg	418	Neg	Neg	6551	Pos/Neg	-
67	A3EYE	N	5266	Neg	Neg	2734	Pos	Pos	486		Neg	9862	Pos/Neg	Pos
68	A3EYE	N	952	Pos/Neg	Neg	680	Pos	Pos	168		Neg	4342	Pos	Pos
69	A3EYE	Y	2607	Pos	Neg	4277	Pos	Pos	200		Neg	9972	Pos	Pos
70	A3EYE	Y	8284	Pos	?	1493	Pos	Pos	392		Pos	689	Pos	Pos

Table 9.2 Comparison of ELISA IBV antibody levels (IDEXX) with SN and AGP, sorted by ELISA antibody titre

IDEXX	AGP	SN
1	Neg	Neg
1	Neg	Neg
1	Neg	Neg
1	Pos	Neg
1	Neg	Neg
1	Neg	Neg
1	Neg	Neg
1	Pos,Pos	Neg
1	Neg	Neg
7	Neg	Neg
9	?	Neg
15	Neg	Neg
24	?	Neg
24	?	Neg
24	?, Pos?	Neg
25	Pos?	Neg
34	?	Neg
39	Neg	Neg
45	Neg	Neg
48	Neg	Neg
50	Neg	Neg
50	?	Neg
54	Neg	Neg
63	Neg	Neg
67	Neg	Pos
67	Neg	Neg
69	Neg	Pos/Neg
69	Neg	Neg
74	Neg	Neg
86	Neg	Neg
86	Neg	Pos
95	Neg	Neg
97	?	Pos/Neg
103	?	Neg
103	Neg	Neg
103	Pos	Pos/Neg
105	Pos	Neg
109	Neg	Neg
114	Neg	Neg
114	Neg	Pos/Neg
114	Pos	Pos/Neg
114	Neg	Neg
132	Neg	Pos/Neg
133	?	Pos
138	Neg	Neg
138	Pos	Neg
138	Neg	Neg

IDEXX	AGP	SN
138	?	Pos
138	?	Pos
142	?	Neg
142	Neg	Neg
150	Neg	Neg
150	Pos	Neg
150	Neg	Pos/Neg
152	Neg	Neg
156	?	Pos
156	Neg	Neg
156	Pos	Pos
160	Neg,Neg	Neg
168	Neg	
172	Neg	Neg
172	Neg	Pos
176	Pos	Pos
182	Pos	Neg
182	Neg	Pos
182	Pos	Neg
192	Neg	Neg
200	Pos	Neg
200	Neg	
207	Neg	Neg
207	Neg	Neg
219	?	Neg
219	Pos	Neg
219	Pos	Pos
225	Neg	Neg
229	Neg	Pos
242	Neg	Neg
244	Pos	Pos/Neg
244	Pos	Pos
250	Neg	Neg
252	Pos	Pos
254	Neg	Pos/Neg
262	Neg	Neg
279	Pos	Neg
288	Pos	Pos
298	Neg	Pos
300	Neg	Neg
300	Neg	Neg
302	Neg	Neg
313	Neg	Neg
319	Neg	Neg
321	?	Pos/Neg
324	Neg	Pos
330	Neg	Pos

Table 9.2 (cont)

IDEXX	AGP	SN
334	Neg	Pos
334	Pos	Neg
343	Neg	Pos
349	Neg	Pos
360	Neg	Neg
360	Neg	Neg
360	Pos	Neg
366	Neg	Pos
366	?	Neg
383	Neg	Neg
392	Pos	
401	Neg	Neg
405	Neg	Neg
412	Pos	Pos
416	Neg	Pos
418	Neg	Neg
427	Neg	Neg
431	Neg	Pos/Neg
444	Neg	Neg
444	Pos	Pos
457	?	Neg
466	Pos	Pos/Neg
477	Pos	Pos/Neg
486	Neg	
490	?	Pos
510	Neg	Neg
510	Neg	Neg
510	Neg	Neg
541	Neg	
552	Pos	Neg
592	Pos	Neg
608	Neg	Pos
612	Neg	Neg
614	?	Pos
628	Neg	Pos/Neg
635	Neg	Pos
646	Pos	Pos
668	Pos	Pos
673	Neg	Neg
673	Neg	Neg
680	Pos	Pos
689	Pos	Pos
711	Neg	Neg
718	Neg	Neg
739	Neg	Pos
796	Pos	Pos
800	Pos?	Pos
823	Pos	Pos/Neg
828	Pos	Pos

IDEXX	AGP	SN
835	Pos	Pos
851	Neg	Pos
852	Neg	Pos/Neg
855	Pos	Pos
904	Pos	Pos
906	Neg	Neg
959	?	Pos/Neg
964	Neg	Neg
973	Neg	Neg
976	Pos	Neg
985	Neg	Neg
985	Pos	Neg
997	Neg/Pos	Pos
999	Pos	Neg
1008	Neg	Pos
1039	Pos	Pos
1041	Neg	Neg
1046	Pos	Pos
1067	Neg	Pos
1076	Neg	Neg
1090	Pos	Neg
1114	Neg	Pos
1121	Neg	Pos
1133	Neg	Neg
1180	Neg?	Neg
1199	Neg	Pos
1318	Neg	Neg
1368	Neg	Neg
1372	?	Pos
1387	Pos	Pos
1399	?,?	Pos/Neg
1454	Neg	Pos
1469	Neg	Pos
1481	Neg	Pos
1493	Pos	Pos
1522	Pos	Pos/Neg
1584	Pos	Pos
1640	Pos	Pos
1694	Neg	Pos
1730	Neg	Pos
1738	Pos	Pos
1747	Neg?	Neg
1774	Neg	Pos
1777	Pos	Pos
1786	Pos	Pos
1818	Neg	Neg
1882	Neg	Neg
1919	Neg	Neg
1983	Pos	Pos

Table 9.2 (cont)

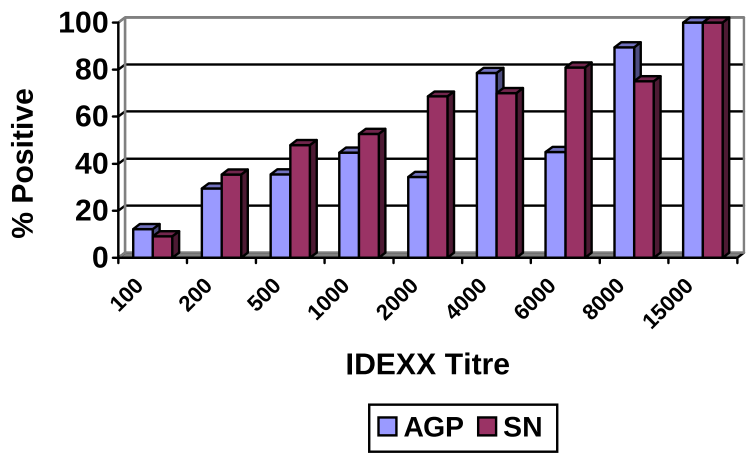
IDEXX	AGP	SN
2025	Neg	Neg
2134	Neg	
2283	Pos	Pos
2388	Pos	Pos
2461	Neg	Neg
2542	Neg	Pos
2582	Neg	Pos
2607	Neg	Pos
2620	Pos	Pos
2663	Pos	Pos
2734	Pos	Pos
2745	Neg	Neg
2746	Pos	Pos
2883	Neg	Neg
2889	Neg	Pos
2978	Pos	Pos
3054	-	Pos
3085	Pos	Pos/Neg
3126	Pos	Neg
3157	Pos	Neg
3165	Pos	Pos
3208	Pos	Pos
3247	Neg	Neg
3308	Pos	Pos
3319	-	Pos
3388	??	Pos
3404	Pos	Pos
3740	Pos	Pos
3821	Pos	Pos
3847	Pos	Neg
4033	Neg	Neg
4056	Pos	Neg
4277	Pos	Pos
4300	Pos	Pos
4342	Pos	Pos
4363	Neg	Pos
4482	Neg	Pos/Neg
4530	Neg	Pos
4532	Pos	Pos
4670	Neg	Neg
4696	Neg	Pos

IDEXX	AGP	SN
4903	Pos	Pos
5007	?,neg	Pos
5108	Pos	Pos
5266	Neg	Neg
5408	-	Neg
5529	Pos	Pos
5717	Pos	Pos
5757	Neg	Pos
5825	Neg	Neg
5889	?	Pos
6092	Pos	Pos
6100	Pos	Pos
6206	Pos	Pos
6404	Pos	Pos
6434	Pos	Pos
6518	Pos	Pos
6551	-	Pos/Neg
7400	Pos	Pos
7463	Pos	Pos
7689	Pos	Pos
7946	Pos	
8123	Pos	Neg
8145	Pos	Pos
8284	?	Pos
8323	Pos	Neg
8498	Pos	Neg
8540	Pos	Pos
8693	Pos	Pos/Neg
8844	Pos	Pos
9300	Pos	Pos
9862	Pos	Pos/Neg
9972	Pos	Pos
10082	Pos	Pos
10364	-	No
10647	Pos	Pos
10971	Pos	Pos
10991	Pos	Pos
11196	Pos	Pos
11315	Pos	
11763	Pos	Pos
14253	Pos	Pos

Table 9.3 Percentage of positive samples (%) as determined by IDEXX ELISA, AGP or SN testing

Age of Hens (weeks)	% Positive by IDEXX ELISA	% Positive by AGP	% Positive by SN
6	70.0	13.0	37.1
16	67.1	45.7	44.3
58	24.3	38.6	30.4
80	85.9	71.2	77.1

Figure 9.4 Comparison of IBV antibody titres obtained with IDEXX ELISA kit and percentage of samples in the titre range positive by AGP or SN test



9.5 Discussion and Conclusions

The different methods of measuring IBV antibody levels probably measure different types of antibodies. Therefore, it is not surprising that the results obtained are not identical. When birds were vaccinated during rearing, there was an antibody response, measured by ELISA, AGP and SN, on a group basis. However, ELISA was more sensitive in detecting the antibody response at 6 and 16 weeks of age. A preference for ELISA has been expressed by authors from previous studies (Mockett & Darbyshire, 1981; Monreal *et al.*, 1983; de Wit *et al.*, 1992). However, the value of the greater sensitivity of ELISA is equivocal in relation to the resistance of the birds to intercurrent infection.

Further information is required to address the need for better surveillance against IBV in layer flocks.

Appendix A: Preliminary Trial

Table A1 Body weight (g) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages
Control	1178.50[E] ±50.6	1523.75[D] ±25.6	1672.07[C] ±24.9	1642.60[CD] ±92.4	1935.40[B] ±39.4	2308.00[A] ±71.8	1575.11[c] ±38.9
Eyedrop at day-old	1255.25 ±35.4	1550.00 ±51.5	1696.30 ±20.3	1717.80 ±70.5			1640.25[b] ±27.4
Eyedrop at 2 weeks	1300.50 ±29.5	1319.25 ±45.8	1624.92 ±28.9	1837.60 ±60.0			1587.56[c] ±33.0
Coarse spray at day-old	1278.50 ±41.60	1637.50 ±35.4	1785.81 ±12.6	1964.00 ±41.0			1741.41[a] ±30.4
Coarse spray at 2 weeks	1181.00 ±20.7	1426.25 ±90.9	1628.64 ±27.16	1803.80 ±34.3			1583.26[c] ±33.8
In water at day-old	1296.50[d] ±42.6	1391.50[c] ±27.9	1629.41[b] ±23.7	1956.40[a] ±37.1			1615.00b[c] ±30.2
In water at 2 weeks	1211.25 ±36.3	1426.25 ±64.14	1622.16 ±24.3	1939.60 ±23.8			1600.61[bc] ±34.6
All groups	1243.07[d] ±15.7	1467.79[c] ±26.2	1664.37[b] ±9.8	1837.40[a] ±27.6			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
Significant effect of treatment group ($P<0.0001$)
Significant interaction between stage and treatment group ($P=0.0003$)
For control only, significant effect of stage of experiment (weeks) ($P<0.0001$)

Table A2 Total kidney weight (% body weight) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	Wks 9-12
Control	0.96^{AB} ±0.05	0.91^{AB} ±0.06	0.79^{B} ±0.02	1.14^{A} ±0.2	0.86^{B} ±0.03	0.86^{B} ±0.02	0.90^{a} ±0.05
Eyedrop at day-old	1.01 ±0.09	0.79 ±0.03	0.78 ±0.01	0.78 ±0.05			$0.81b^{c}$ ±0.02
Eyedrop at 2 weeks	0.96 ±0.06	0.86 ±0.05	0.77 ±0.02	0.84 ±0.03			$0.81b^{c}$ ±0.02
Coarse spray at day-old	0.91 ±0.03	0.81 ±0.04	0.78 ±0.02	0.78 ±0.04			0.80^{cd} ±0.01
Coarse spray at 2 weeks	0.87 ±0.04	0.82 ±0.02	0.80 ±0.02	0.90 ±0.04			$0.82b^{c}$ ±0.02
In water at day-old	0.88 ±0.04	0.84 ±0.04	0.73 ±0.02	0.83 ±0.03			0.76^{d} ±0.02
In water at 2 weeks	0.95 ±0.04	0.91 ±0.03	0.81 ±0.02	0.89 ±0.02			0.85^{ab} ±0.02
All groups	0.94^{a} ±0.02	0.85^{b} ±0.02	0.78^{c} ±0.01	0.88^{b} ±0.04			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
Significant effect of treatment group ($P=0.0006$)
Significant interaction between stage and treatment group ($P=0.0042$)
For control only, significant effect of stage of experiment (weeks) ($P=0.0288$)

Table A3 Right kidney weight (% body weight) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages
Control	0.47^{AB} ±0.02	0.42^{B} ±0.03	0.40^{B} ±0.01	0.57^{A} ±0.10	0.43^{B} ±0.01	0.41^{B} ±0.01	0.45^{a} ±0.02
Eyedrop at day-old	0.52 ±0.06	0.38 ±0.02	0.39 ±0.01	0.38 ±0.03			0.40^{bc} ±0.01
Eyedrop at 2 weeks	0.49 ±0.03	0.43 ±0.02	0.38 ±0.01	0.43 ±0.02			0.40^{bc} ±0.01
Coarse spray at day-old	0.46 ±0.03	0.40 ±0.02	0.38 ±0.01	0.40 ±0.02			0.39^{c} ±0.01
Coarse spray at 2 weeks	0.44 ±0.02	0.40 ±0.02	0.40 ±0.01	0.44 ±0.02			0.41^{bc} ±0.01
In water at day-old	0.47 ±0.03	0.42 ±0.03	0.36 ±0.01	0.42 ±0.01			0.38^{c} ±0.01
In water at 2 weeks	0.49 ±0.03	0.46 ±0.02	0.40 ±0.01	0.43 ±0.01			0.42^{ab} ±0.01
All groups	0.48^{a} ±0.01	0.42^{b} ±0.01	0.39^{c} ±0.004	0.44^{b} ±0.02			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
Significant effect of treatment group ($P=0.0309$)
Significant interaction between stage and treatment group ($P=0.0187$)
For control only, significant effect of stage of experiment (weeks) ($P=0.0295$)

Table A4 Left kidney weight (% body weight) of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14	All ages
Control	0.49^{AB} ±0.03	0.49^{AB} ±0.03	0.40^{B} ±0.01	0.57^{A} ±0.11	0.43^{B} ±0.02	0.45^{AB} ±0.01	0.45^{a} ±0.02
Eyedrop at day-old	0.50 ±0.04	0.41 ±0.02	0.39 ±0.01	0.40 ±0.02			0.41^{b} ±0.01
Eyedrop at 2 weeks	0.47 ±0.03	0.43 ±0.03	0.39 ±0.01	0.41 ±0.02			0.40^{b} ±0.01
Coarse spray at day-old	0.45 ±0.03	0.41 ±0.02	0.39 ±0.02	0.38 ±0.02			0.40^{bc} ±0.01
Coarse spray at 2 weeks	0.43 ±0.02	0.42 ±0.02	0.40 ±0.01	0.46 ±0.02			0.41^{b} ±0.01
In water at day-old	0.41 ±0.02	0.41 ±0.02	0.36 ±0.01	0.41 ±0.02			0.38^{c} ±0.01
In water at 2 weeks	0.47 ±0.01	0.45 ±0.02	0.41 ±0.01	0.45 ±0.01			0.42^{ab} ±0.01
All groups	0.46^{a} ±0.01	0.43^{a} ±0.01	0.39^{b} ±0.04	0.44^{a} ±0.02			

Values are Mean ± S.E.
Significant effect of stage of experiment (weeks) ($P<0.0001$)
Significant effect of treatment group ($P=0.0004$)
Significant interaction between stage and treatment group ($P=0.0274$)
For control only, significant effect of stage of experiment (weeks) ($P=0.0245$)

Table A5 IBV antibody titres of cockerels exposed to T-strain IBV following different vaccination protocols

	Week 7	Week 11	Week 12	Week 13	Week 14
Control	0	0	0	5647	9153
Eyedrop at day-old	1251	1612	1604		
Eyedrop at 2 weeks	2106.5	2032	2004		
Coarse spray at day-old	1449.5	2054.5	4939		
Coarse spray at 2 weeks	1238.5	1166.5	1706		
In water at day-old	462	1610.5	2924.5		
In water at 2 weeks	1578.5	1145.5	4054		

Values are Means.

Appendix B: Trial 1 – Rearing Phase

Table B1 Body weight (g) of ISA brown hens exposed to the following different IB vaccination protocols in the rearing phase

	Age of Birds (weeks)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	52.9	102.2	153.6	238.4	334.3 ±7.9	446.0 ±10.7	569.1 ±13.3	694.2 ±15.8	823.7 ±17.7	947.6 ±20.8	1045.8 ±21.6	1178.3 ±22.9	1242.8 ±23.9	1361.8 ±24.7	1456.7 ±31.4
VicS, eyedrop	60.7	105.95	170.4	264.9	377.3 ±7.3	491.9 ±10.1	584.1 ±12.7	691.9 ±14.8	808.80 ±16.7	926.4 ±20.8	1031.5 ±19.2	1138.4 ±20.1	1198.4 ±19.2	1323.9 ±20.5	1421.9 ±20.4
VicS, coarse spray	53.8	103.8	171.0	258.1	372.3 ±5.3	477.5 ±10.7	602.7 ±14.8	715.5 ±16.1	839.9 ±19.7	965.9 ±19.8	1057.6 ±23.7	1194.9 ±25.4	1264.8 ±24.0	1388.0 ±27.1	1484.7 ±26.9
VicS, in water	56.9	90.7	139.0	216.9	328.5 ±9.1	434.8 ±11.2	555.9 ±17.4	668.5 ±15.9	796.0 ±15.5	928.5 ±19.4	1023.1 ±25.3	1161.4 ±24.0	1241.0 ±23.7	1357.2 ±24.4	1451.5 ±25.6
A3, eyedrop at	66.6	117.4	185.9	277.5	362.2 ±7.1	471.9 ±9.2	594.7 ±10.7	698.8 ±11.9	826.6 ±14.8	966.7 ±18.6	1049.2 ±16.6	1173.5 ±19.8	1235.3 ±19.9	1356.5 ±20.1	1439.4 ±23.9
A3, coarse spray	57.4	106.2	179.9	264.0	380.9 ±6.9	503.2 ±7.6	617.3 ±10.8	723.9 ±11.0	849.1 ±14.5	969.6 ±14.2	1060.4 ±18.5	1195.1 ±19.0	1255.1 ±22.8	1382.8 ±22.4	1459.0 ±26.8
A3, in water	61.8	109.2	171.3	259.0	374.0 ±5.7	485.5 ±7.3	613.7 ±9.5	724.3 ±11.6	848.1 ±12.0	978.7 ±11.5	1081.3 ±14.4	1189.0 ±16.7	1264.3 ±15.7	1374.2 ±15.9	1480.4 ±19.2

Values are Mean ± S.E.

Appendix C: Trial 1 – Laying Phase

Table C1 Body weight (g) of ISA brown hens exposed to the following different IB vaccination protocols in the laying phase and **not revaccinated** regularly during lay

Not Revaccinated	Wk 16	Wk 17	Wk 18	Wk 19	Wk 20	Wk 21	Wk 22	Wk 23	Wk 25	Wk 30	Wk 35	Wk 41
Control	1481.9 ±41.7	1599.0 ±40.7	1668.6 ±38.0	1788.1 ±47.6	1800.8 ±42.5	1804.7 ±47.1	1769.6 ±48.3	1806.6 ±49.4	1851.4 ±54.2	1986.3 ±73.5	2022.8 ±69.2	1998.8 ±67.0
VicS, eyedrop at day-old	1496.3 ±29.8	1634.30 ±29.9	1746.4 ±47.0	1818.6 ±56.5	1819.9 ±51.0	1816.1 ±50.0	1801.9 ±39.6	1842.0 ±47.5	1851.7 ±48.5	1957.3 ±55.9	2004.2 ±58.9	2021.1 ±68.5
VicS, coarse spray at dayold	1543.6 ±54.4	1634.9 ±55.1	1720.8 ±53.6	1784.9 ±45.5	1817.8 ±49.6	1757.6 ±61.1	1769.0 ±50.5	1818.0 ±49.0	1806.0 ±52.5	1950.2 ±58.9	1943.3 ±60.8	1908.7 ±67.8
VicS, in water at day-old	1499.0 ±31.3	1614.9 ±27.0	1734.7 ±41.7	1771.7 ±25.6	1833.7 ±20.0	1781.9 ±17.9	1773.9 ±16.9	1834.4 ±27.0	1865.0 ±27.9	2018.0 ±53.8	2025.0 ±59.6	2087.0 ±68.7
A3, eyedrop at day-old	1502.7 ±32.0	1607.2 ±31.1	1671.0 ±24.2	1789.8 ±38.3	1797.0 ±44.3	1796.6 ±45.5	1765.1 ±47.6	1807.1 ±41.1	1853.1 ±47.1	1987.8 ±49.7	2017.4 ±45.4	2014.0 ±49.5
A3, coarse spray at dayold	1475.2 ±34.4	1574.8 ±41.0	1646.4 ±34.2	1733.3 ±36.9	1816.0 ±43.1	1842.5 ±49.5	1774.8 ±49.5	1806.7 ±41.7	1826.4 ±42.4	1947.0 ±46.25	1979.9 ±48.5	1989.4 ±53.1
A3, in water at day-old	1560.5 ±33.6	1652.0 ±43.8	1698.7 ±39.1	1790.4 ±44.9	1835.4 ±47.7	1832.4 ±44.6	1804.2 ±43.4	1830.4 ±54.9	1875.3 ±50.1	1965.7 ±74.9	2046.3 ±56.6	2050.1 ±68.0

Values are Mean ± S.E.

Table C2 Body weight (g) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase and **revaccinated** every 8 weeks during lay

Revaccinated	Wk 16	Wk 17	Wk 18	Wk 19	Wk 20	Wk 21	Wk 22	Wk 23	Wk 25	Wk 30	Wk 35	Wk 41
Control	1564.6 ±37.6	1667.7 ±35.3	1767.1 ±36.9	1776.5 ±44.7	1841.0 ±44.3	1743.7 ±51.1	1830.9 ±49.9	1908.6 ±63.9	1903.7 ±68.2	2113.1 ±73.3	2059.2 ±51.0	2129.7 ±58.6
VicS, eyedrop at day-old	1492.1 ±28.5	1584.6 ±32.4	1680.7 ±45.0	1735.5 ±44.7	1790.0 ±49.1	1682.4 ±55.4	1745.5 ±55.5	1796.6 ±54.9	1803.3 ±51.6	1990.6 ±63.9	2042.3 ±106.6	2150.6 ±136.6
VicS, coarse spray at day-old	1621.6 ±33.7	1686.3 ±34.2	1760.7 ±32.10	1819.6 ±30.0	1910.9 ±44.1	1860.8 ±30.8	1882.6 ±28.9	1891.6 ±43.8	1931.6 ±43.8	2056.3 ±78.3	2149.4 ±49.1	2217.2 ±60.9
VicS, in water at day-old	1561.2 ±35.1	1650.4 ±33.6	1710.5 ±28.2	1796.9 ±24.1	1798.3 ±36.8	1802.4 ±31.0	1789.1 ±37.5	1849.4 ±45.2	1841.5 ±40.2	2018.2 ±44.9	2056.0 ±44.6	2068.3 ±37.9
A3, eyedrop at day-old	1551.9 ±38.7	1614.0 ±33.8	1733.3 ±36.1	1791.5 ±37.2	1833.7 ±38.7	1816.5 ±44.2	1841.0 ±42.5	1868.9 ±43.9	1916.0 ±49.4	2085.4 ±59.4	2040.9 ±56.71	2083.0 ±61.5
A3, coarse spray at day-old	1590.2 ±36.5	1668.9 ±27.6	1713.0 ±27.6	1759.4 ±24.2	1815.9 ±46.3	1816.1 ±35.0	1842.1 ±34.5	1848.9 ±46.3	1859.0 ±45.8	2028.8 ±49.7	2029.1 ±53.9	2080.0 ±39.2
A3, in water at day-old	1585.6 ±31.5	1696.0 ±32.4	1731.8 ±46.3	1798.0 ±52.0	1818.3 ±52.0	1834.0 ±73.2	1832.1 ±64.5	1843.8 ±49.6	1866.4 ±53.7	2100.0 ±55.6	2117.3 ±57.6	2149.3 ±50.0

Values are Mean ± S.E.

Table C3. Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

Not revaccinated	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Control	4.04	14.29	26.13 ±6.9	58.15 ±4.9	76.31 ±4.7	92.02 ±3.5	95.77 ±2.6	96.37 ±1.7	95.18 ±1.2	96.25 ±1.3	95.83 ±1.4	94.76 ±1.7	92.86 ±3.3	89.34 ±3.8
VicS, eyedrop at day-old	5.90	23.29	45.12 ±5.9	68.33 ±2.0	82.86 ±4.1	89.05 ±3.6	94.23 ±1.6	92.86 ±3.0	91.96 ±2.6	91.85 ±4.0	93.45 ±4.0	92.86 ±2.5	91.13 ±5.0	93.99 ±3.3
VicS, coarse spray at day-old	4.97	29.19	50.30 ±6.6	64.46 ±6.0	86.25 ±3.4	88.39 ±3.5	96.13 ±1.6	96.55 ±0.8	94.70 ±0.3	96.01 ±2.0	97.44 ±0.8	98.87 ±1.3	97.14 ±1.1	95.89 ±1.0
VicS, in water at day-old	8.70	22.05	43.33 ±6.1	58.40 ±4.4	72.38 ±4.0	83.97 ±4.1	87.71 ±3.6	90.11 ±3.2	95.48 ±3.0	94.71 ±0.4	96.76 ±2.1	94.57 ±2.3	97.26 ±1.2	94.57 ±1.6
A3, eyedrop at day-old	2.80	16.16	28.75 ±3.2	51.25 ±4.6	70.24 ±5.2	86.25 ±2.5	90.12 ±3.4	95.95 ±1.4	100.45 ±4.0	96.98 ±1.5	94.96 ±1.8	95.75 ±1.8	96.43 ±2.2	94.12 ±1.8
A3, coarse spray at day-old	1.55	9.00	30.89 ±6.7	45.00 ±11.6	67.86 ±6.3	83.45 ±6.3	93.69 ±0.8	94.58 ±2.1	92.62 ±0.6	95.95 ±2.0	93.93 ±1.4	95.65 ±0.7	95.48 ±1.3	94.64 ±0.8
A3, in water at day-old	8.16	22.45	44.23 ±5.7	56.84 ±6.4	80.51 ±1.9	88.31 ±2.3	96.99 ±1.3	96.75 ±2.3	94.66 ±3.1	95.42 ±2.1	94.00 ±1.5	96.28 ±1.2	96.08 ±1.1	95.69 ±1.3

Values are Mean ± S.E.

Table C3 (cont). Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

Not revaccinated	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Control	86.25 ±3.0	77.202 ±11.3	79.05 ±11.0	93.40 ±3.1	91.79 ±1.7	94.29 ±1.7	95.18 ±1.6	90.48 ±1.9	96.13 ±1.2	90.42 ±1.4	90.00 ±3.3	88.81 ±0.8	85.30 ±2.5	85.00 ±2.8
VicS, eyedrop at day-old	90.18 ±2.3	84.58 ±5.2	81.82 ±5.0	93.68 ±3.4	88.14 ±2.6	92.92 ±2.3	95.04 ±2.8	95.71 ±1.7	95.48 ±1.4	95.18 ±1.2	93.15 ±2.9	90.91 ±2.0	89.17 ±1.4	91.01 ±1.4
VicS, coarse spray at day-old	91.37 ±3.3	87.70 ±1.5	86.31 ±3.6	98.96 ±2.1	96.13 ±2.0	96.03 ±0.8	98.42 ±2.4	98.52 ±2.4	94.48 ±0.8	95.54 ±1.5	91.47 ±1.7	94.25 ±1.5	90.68 ±1.7	92.56 ±2.5
VicS, in water at day-old	90.52 ±1.8	82.67 ±2.4	75.62 ±9.1	95.95 ±2.8	96.29 ±1.4	95.07 ±1.8	92.85 ±2.4	92.54 ±2.3	91.33 ±2.4	94.83 ±2.1	91.14 ±2.8	90.79 ±2.7	90.41 ±1.4	93.35 ±2.7
A3, eyedrop at day-old	87.73 ±1.6	85.52 ±2.8	86.72 ±4.7	94.43 ±2.2	92.08 ±4.1	90.78 ±4.2	91.43 ±4.0	91.07 ±4.2	90.36 ±5.06	89.03 ±5.04	90.37 ±3.6	88.67 ±3.8	87.44 ±2.7	87.55 ±4.2
A3, coarse spray at day-old	86.96 ±3.3	81.49 ±2.7	82.86 ±4.6	96.18 ±1.5	90.38 ±2.1	91.67 ±3.0	90.48 ±4.0	89.70 ±5.3	86.19 ±3.9	88.75 ±3.4	86.79 ±4.6	87.98 ±4.5	83.39 ±3.6	85.77 ±2.0
A3, in water at day-old	90.66 ±5.5	78.31 ±2.3	78.43 ±6.4	94.97 ±2.1	95.78 ±1.0	96.14 ±0.7	94.59 ±1.4	94.95 ±0.8	97.50 ±3.0	94.12 ±2.4	94.12 ±1.1	95.99 ±1.2	88.62 ±2.0	93.26 ±1.1

Values are Mean ± S.E.

Table C3 (cont). Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

Not revaccinated	44	45	46	47	48	49	50	51	52	53	54	55	56
Control	77.26 ±2.3	85.95 ±0.9	84.29 ±1.3	82.56 ±2.3	84.70 ±4.8	88.14 ±4.9	83.01 ±2.8	88.21 ±3.9	82.92 ±4.6	81.61 ±3.7	74.18 ±7.5	88.45 ±9.9	83.67 ±6.6
VicS, eyedrop at day-old	91.31 ±0.7	90.57 ±4.1	87.72 ±3.7	92.75 ±2.8	91.25 ±5.0	92.02 ±4.6	87.48 ±2.9	93.56 ±2.2	86.03 ±3.8	86.09 ±4.9	85.90 ±4.8	95.02 ±7.2	88.08 ±2.8
VicS, coarse spray at day-old	87.90 ±3.4	89.38 ±2.6	87.30 ±2.0	89.88 ±2.5	94.54 ±3.9	91.47 ±5.6	89.09 ±4.3	89.86 ±7.4	89.82 ±8.0	91.87 ±5.5	84.88 ±3.8	90.78 ±5.1	85.62 ±3.6
VicS, in water at day-old	88.06 ±2.9	89.22 ±2.2	85.24 ±7.1	90.52 ±1.7	92.68 ±2.9	91.15 ±1.9	88.39 ±1.2	89.79 ±4.0	84.18 ±6.3	84.38 ±4.5	86.47 ±3.9	89.89 ±3.3	84.38 ±3.6
A3, eyedrop at day-old	88.24 ±1.6	93.94 ±2.5	84.99 ±5.1	90.62 ±3.9	89.61 ±6.2	89.75 ±5.1	87.74 ±5.0	89.97 ±3.6	86.44 ±4.6	89.97 ±3.6	77.02 ±6.2	86.08 ±5.5	81.47 ±5.4
A3, coarse spray at day-old	83.87 ±1.0	89.75 ±1.0	83.80 ±2.4	84.72 ±1.9	84.32 ±3.4	87.17 ±1.6	88.41 ±2.8	87.52 ±1.5	85.51 ±1.0	79.26 ±2.5	79.92 ±2.8	86.80 ±2.8	85.42 ±1.9
A3, in water at day-old	90.52 ±1.0	91.38 ±2.4	93.17 ±0.8	91.31 ±2.5	91.89 ±1.7	96.26 ±2.6	91.01 ±4.2	90.31 ±3.4	86.09 ±5.0	87.03 ±2.8	84.05 ±1.6	91.30 ±2.1	90.08 ±1.7

Values are Mean ± S.E.

Table C4 Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

Revaccinated	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Control	2.17	9.01	24.58 ±7.9	57.20 ±5.9	79.82 ±3.4	87.08 ±4.1	90.83 ±2.3	89.40 ±3.2	86.90 ±4.4	85.95 ±4.5	86.84 ±4.8	87.56 ±2.8	89.28 ±1.8	89.21 ±1.8
VicS, eyedrop at day-old	0.93	6.21	21.55 ±2.4	51.43 ±4.4	73.99 ±2.8	81.96 ±5.5	91.25 ±2.8	88.63 ±6.3	87.80 ±3.3	85.61 ±3.7	90.38 ±3.5	89.48 ±5.0	91.52 ±3.6	95.09 ±2.6
VicS, coarse spray at day-old	2.17	14.60	29.29 ±8.3	59.46 ±4.9	81.96 ±5.1	88.01 ±2.7	92.64 ±1.2	91.45 ±2.4	85.73 ±5.1	92.45 ±1.9	91.05 ±2.8	91.97 ±4.1	94.53 ±2.0	94.29 ±3.7
VicS, in water at day-old	1.55	10.56	34.05 ±8.1	64.31 ±6.4	79.11 ±6.5	85.89 ±2.5	90.36 ±2.8	90.83 ±2.6	97.5 ±2.4	92.75 ±0.6	95.69 ±3.0	96.98 ±1.1	96.05 ±1.3	97.45 ±1.0
A3, eyedrop at day-old	3.73	11.18	31.79 ±1.7	62.14 ±5.04	81.49 ±1.5	88.87 ±0.8	92.08 ±1.9	94.82 ±1.6	93.93 ±1.7	86.96 ±2.6	96.61 ±3.5	95.18 ±1.2	94.40 ±2.5	97.62 ±1.7
A3, coarse spray at day-old	4.97	23.60	39.94 ±3.1	65.59 ±1.9	80.36 ±4.0	88.33 ±2.6	92.09 ±2.6	89.20 ±3.9	88.58 ±3.5	83.42 ±3.2	88.00 ±4.9	90.26 ±2.8	93.15 ±1.1	96.25 ±2.6
A3, in water at day-old	5.44	21.09	36.13 ±5.4	68.96 ±5.1	84.79 ±7.7	88.18 ±2.6	89.67 ±1.5	91.87 ±1.9	85.95 ±4.1	86.81 ±4.9	91.99 ±2.0	93.69 ±3.9	92.74 ±2.0	94.14 ±2.9

Values are Mean ± S.E.

Table C4 (cont). Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

Revaccinated	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Control	86.79 ±2.2	90.24 ±3.8	90.89 ±4.0	91.32 ±1.3	90.71 ±1.3	92.98 ±2.8	89.05 ±3.0	88.51 ±2.2	90.36 ±1.0	90.83 ±1.8	92.02 ±1.3	88.45 ±2.5	86.73 ±1.2	86.96 ±1.3
VicS, eyedrop at day-old	92.71 ±3.1	93.15 ±3.7	92.11 ±3.1	92.01 ±4.5	90.62 ±5.9	93.32 ±3.9	90.35 ±3.5	91.24 ±3.4	92.09 ±1.5	89.97 ±2.6	90.56 ±2.7	90.73 ±3.9	88.05 ±3.0	88.73 ±4.0
VicS, coarse spray at day-old	92.00 ±3.7	89.56 ±4.0	87.75 ±5.9	91.12 ±3.1	93.07 ±3.0	93.99 ±3.1	90.95 ±4.4	93.13 ±3.1	92.86 ±5.0	90.12 ±5.5	93.39 ±2.8	85.71 ±3.9	87.34 ±3.5	87.14 ±5.9
VicS, in water at day-old	95.82 ±0.9	97.78 ±0.9	94.92 ±2.4	93.58 ±3.4	93.76 ±3.3	93.33 ±1.9	92.67 ±2.2	91.11 ±2.1	91.07 ±2.7	90.99 ±1.9	90.32 ±3.7	90.04 ±1.1	86.72 ±3.0	91.49 ±2.6
A3, eyedrop at day-old	97.62 ±1.4	94.10 ±0.5	92.98 ±1.6	96.81 ±1.0	94.88 ±1.5	94.46 ±0.9	94.64 ±1.8	95.54 ±2.0	95.77 ±1.0	93.69 ±1.7	90.89 ±2.7	92.32 ±1.2	92.14 ±3.1	90.12 ±2.1
A3, coarse spray at day-old	94.88 ±2.5	95.02 ±1.6	92.68 ±1.3	89.52 ±7.9	93.79 ±1.2	92.86 ±2.7	90.98 ±1.9	91.56 ±2.7	93.65 ±1.6	92.39 ±3.4	91.09 ±2.9	91.88 ±2.4	88.24 ±1.5	89.14 ±3.0
A3, in water at day-old	95.06 ±2.9	91.01 ±4.3	91.01 ±4.3	91.67 ±3.4	91.25 ±2.6	92.50 ±4.0	92.50 ±4.0	93.48 ±2.6	93.84 ±3.0	92.38 ±3.4	93.12 ±2.5	91.73 ±2.3	90.30 ±3.2	86.61 ±3.7

Values are Mean ± S.E.

Table C4 (cont). Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

Revaccinated	44	45	46	47	48	49	50	51	52	53	54	55	56
Control	87.97 ±2.1	86.73 ±2.8	88.21 ±1.7	86.79 ±2.7	86.37 ±2.8	84.16 ±2.7	85.12 ±3.2	84.05 ±6.5	83.15 ±3.6	82.56 ±3.5	81.31 ±3.1	77.27 ±4.0	78.81 ±3.5
VicS, eyedrop at day-old	86.82 ±4.6	85.84 ±3.7	84.86 ±3.2	91.07 ±1.8	88.39 ±2.3	88.20 ±4.5	86.74 ±3.1	88.91 ±3.4	91.04 ±3.9	90.75 ±2.2	86.96 ±4.3	86.20 ±7.1	85.15 ±4.2
VicS, coarse spray at day-old	88.67 ±3.7	85.88 ±3.9	84.16 ±2.9	84.51 ±3.6	82.08 ±2.2	86.65 ±2.3	84.87 ±2.9	79.03 ±4.5	85.53 ±3.1	89.48 ±2.6	84.78 ±1.9	86.54 ±1.9	86.67 ±3.6
VicS, in water at day-old	88.03 ±4.9	91.07 ±2.7	91.01 ±4.1	92.32 ±3.7	88.89 ±3.3	87.62 ±3.1	88.59 ±2.7	85.10 ±3.7	84.15 ±2.5	85.52 ±7.6	82.00 ±5.7	78.61 ±6.6	76.71 ±5.2
A3, eyedrop at day-old	91.01 ±2.3	86.96 ±2.4	87.56 ±3.7	90.83 ±2.3	85.95 ±3.7	89.11 ±2.7	87.02 ±1.6	87.26 ±3.1	86.61 ±2.5	85.89 ±3.5	86.43 ±1.9	82.92 ±2.9	90.66 ±1.7
A3, coarse spray at day-old	90.80 ±0.5	87.92 ±2.5	92.35 ±3.6	88.17 ±2.5	85.21 ±3.5	88.76 ±1.8	86.82 ±2.8	86.46 ±3.1	85.25 ±5.2	88.58 ±3.9	86.65 ±3.2	80.32 ±3.2	80.37 ±5.7
A3, in water at day-old	87.92 ±2.0	90.32 ±2.3	88.72 ±3.1	86.67 ±4.5	85.30 ±5.9	89.29 ±1.3	87.62 ±3.5	85.60 ±3.9	87.53 ±5.3	84.70 ±4.3	82.50 ±5.2	84.79 ±3.6	76.01 ±5.5

Values are Mean ± S.E.

Table C5 Feed intake (g/day) of ISA brown following IB revaccination in different IB vaccination protocols in laying phase

	Age (week) of hens						
	18 wks	19 wks	20 wks	30 wks	36 wks	40 wks	48 wks
Not revaccinated	117.30	115.70	129.30	139.12	152.61	173.66	156.97
Control	120.80	124.10	135.45	139.03	153.03	176.70	144.46
VicS, eyedrop at day-old	116.80	114.10	123.08	145.98	146.78	170.98	147.06
VicS, coarse spray at day-old	121.00	117.90	134.70	133.80	150.83	184.50	160.56
VicS, in water at day-old	126.70	120.30	131.83	149.98	152.73	184.42	164.54
A3, eyedrop at day-old	117.50	118.70	134.22	147.30	157.38	164.48	168.70
A3, coarse spray at day-old	100.70	94.63	107.55	127.88	150.38	161.10	152.54
A3, in water at day-old	117.40	120.60	138.29	128.05	158.43	173.66	161.70
Revaccinated	124.60	142.3	141.90	164.81	159.30	191.12	175.15
Control	135.30	136.80	141.54	180.13	189.13	217.22	186.38
VicS, eyedrop at day-old	122.50	141.50	142.92	190.25	166.55	199.05	188.58
VicS, coarse spray at day-old	127.50	133.50	149.10	172.28	168.85	189.77	174.82
VicS, in water at day-old	113.20	135.20	138.63	148.20	149.23	177.00	158.38
A3, eyedrop at day-old	136.50	168.40	143.97	169.68	142.88	186.87	172.62
A3, coarse spray at day-old	116.70	137.80	135.02	150.55	146.35	181.37	179.92
A3, in water at day-old	119.60	142.90	142.14	142.63	152.13	186.52	165.32

Table C6 Egg quality egg weight (g) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	46.77 ±0.5	54.38 ±0.4	58.58 ±0.4	57.91 ±0.4	61.90 ±0.3	61.85 ±0.3	63.04 ±0.4	63.61 ±0.4	62.70 ±0.4	63.36 ±0.4	59.45 ±0.2
Control	45.75 ±0.7	53.47 ±0.9	57.69 ±0.9	56.52 ±0.9	62.04 ±0.9	61.05 ±0.8	62.91 ±0.9	62.38 ±1.1	62.23 ±1.2	61.94 ±1.1	58.60 ±0.5
VicS, eyedrop at day-old	47.15 ±1.4	55.19 ±0.9	61.63 ±2.4	58.06 ±1.3	61.79 ±1.0	61.76 ±1.0	62.77 ±0.9	63.54 ±1.1	63.56 ±1.1	63.96 ±1.0	60.00 ±0.5
VicS, coarse spray at day-old	47.42 ±1.2	54.50 ±0.6	58.46 ±0.7	56.40 ±1.1	61.15 ±0.7	61.01 ±0.9	62.70 ±0.9	65.27 ±0.7	62.69 ±0.8	64.15 ±0.8	59.38 ±0.4
VicS, in water at day-old	47.32 ±1.0	55.34 ±1.2	58.47 ±0.9	58.59 ±0.9	62.60 ±0.7	63.02 ±0.7	62.95 ±0.7	62.85 ±1.0	62.15 ±1.0	63.25 ±0.9	59.66 ±0.4
A3, eyedrop at day-old	46.06 ±0.5	53.93 ±0.9	57.49 ±0.8	59.17 ±1.0	61.84 ±0.9	61.21 ±1.1	63.64 ±1.2	63.97 ±1.1	63.68 ±1.2	64.15 ±0.9	59.64 ±0.5
A3, coarse spray at day-old	48.30 ±2.0	53.79 ±1.4	58.77 ±0.7	60.29 ±0.9	62.89 ±1.0	64.01 ±0.7	64.53 ±0.9	64.45 ±0.9	63.41 ±1.0	62.75 ±2.0	60.32 ±0.5
A3, in water at day-old	45.29 ±1.2	54.45 ±1.1	57.56 ±0.6	56.32 ±1.1	61.02 ±0.8	60.89 ±0.8	61.79 ±0.9	62.79 ±0.7	61.14 ±0.7	63.29 ±0.9	58.52 ±0.4
All Groups	46.77 ±0.5	54.38 ±0.4	58.58 ±0.4	57.91 ±0.4	61.90 ±0.3	61.85 ±0.3	63.04 ±0.4	63.61 ±0.4	62.70 ±0.4	63.36 ±0.4	

Values are Mean ± S.E.

Table C6 (cont). Egg quality egg weight (g) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	47.68 ±0.5	54.64 ±0.5	59.60 ±0.5	61.75 ±0.4	62.76 ±0.4	63.04 ±0.4	64.00 ±0.4	64.21 ±0.4	62.77 ±0.4	63.75 ±0.4	60.45 ±0.2
Control	46.40 ±1.1	53.37 ±0.9	57.79 ±0.9	60.17 ±0.8	61.01 ±0.8	62.06 ±1.3	64.36 ±1.4	62.18 ±0.6	60.36 ±0.9	61.61 ±1.1	58.99 ±0.5
VicS, eyedrop at day-old	47.31 ±0.8	56.81 ±1.9	58.52 ±0.7	61.42 ±0.9	61.81 ±1.0	62.54 ±0.9	63.67 ±1.0	64.20 ±1.1	63.52 ±0.9	63.04 ±1.1	60.29 ±0.5
VicS, coarse spray at day-old	48.28 ±1.5	53.21 ±1.1	60.64 ±1.8	63.39 ±1.7	63.14 ±1.0	63.37 ±1.3	63.59 ±1.1	63.83 ±1.2	62.67 ±1.0	65.26 ±1.4	60.74 ±0.6
VicS, in water at day-old	48.45 ±1.1	53.45 ±0.9	60.17 ±2.1	60.11 ±0.9	63.60 ±1.2	61.94 ±0.9	64.26 ±1.0	64.75 ±1.3	62.66 ±1.2	62.44 ±1.2	60.18 ±0.5
A3, eyedrop at day-old	46.65 ±1.0	55.42 ±1.9	60.16 ±0.9	62.49 ±0.9	63.16 ±0.9	62.91 ±1.0	64.04 ±1.0	65.24 ±1.0	63.66 ±1.1	65.20 ±1.0	60.89 ±0.5
A3, coarse spray at day-old	45.57 ±0.8	55.83 ±1.3	59.17 ±0.7	61.95 ±0.8	62.82 ±0.7	64.17 ±0.7	63.60 ±0.7	63.22 ±0.6	62.74 ±0.7	63.27 ±0.8	60.24 ±0.5
A3, in water at day-old	51.42 ±1.8	54.41 ±0.8	60.73 ±0.9	62.71 ±0.9	63.82 ±1.1	64.29 ±1.5	64.49 ±1.0	66.08 ±1.4	63.77 ±1.3	65.45 ±1.5	61.82 ±0.5
All Groups	47.68 ±0.5	54.64 ±0.5	59.60 ±0.5	61.75 ±0.4	62.77 ±0.4	63.04 ±0.4	64.00 ±0.4	64.22 ±0.4	62.77 ±0.4	63.75 ±0.5	

Values are Mean ± S.E.

Significant difference within shed (<0.0001), within initial treatment (0.0001), ages (<0.0001); Interaction between shed and treatments (<0.0001), between shed and ages (<0.00010), between treatment and ages (0.0004)

Table C7 Egg quality shell reflectivity (%) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	32.47 ±0.5	28.92 ±0.4	31.32 ±0.4	31.95 ±0.4	32.86 ±0.3	35.58 ±0.4	35.98 ±0.4	35.96 ±0.4	36.32 ±0.4	32.98 ±0.4	33.44 ±0.1
Control	34.43 ±1.6	29.38 ±0.8	32.71 ±1.0	32.38 ±0.9	34.14 ±0.9	35.95 ±1.2	36.19 ±1.2	36.09 ±1.1	36.62 ±1.2	33.86 ±0.7	34.18 ±0.4
VicS, eyedrop at day-old	33.20 ±1.1	28.76 ±1.0	33.10 ±1.2	32.29 ±1.0	33.91 ±0.7	35.57 ±0.8	36.24 ±1.0	36.81 ±1.0	36.19 ±0.7	33.14 ±0.7	33.92 ±0.3
VicS, coarse spray at day-old	34.71 ±1.1	29.90 ±1.3	32.19 ±1.2	32.81 ±1.0	33.62 ±1.0	37.29 ±1.4	38.10 ±1.1	37.90 ±1.1	38.81 ±1.2	35.33 ±1.1	35.07 ±0.4
VicS, in water at day-old	31.29 ±1.3	29.24 ±0.9	31.00 ±0.7	31.86 ±1.0	32.62 ±0.8	36.57 ±0.9	34.76 ±0.8	34.90 ±0.8	34.67 ±1.2	30.52 ±0.9	32.74 ±0.3
A3, eyedrop at day-old	30.63 ±1.3	28.10 ±0.9	29.81 ±0.7	30.76 ±0.6	30.91 ±0.7	33.95 ±0.7	35.29 ±0.8	34.95 ±0.9	35.05 ±0.9	31.71 ±1.0	32.13 ±0.3
A3, coarse spray at day-old	32.00 ±1.5	28.52 ±0.7	30.33 ±1.1	31.10 ±1.2	32.62 ±1.2	35.05 ±1.4	35.33 ±1.0	35.29 ±1.3	37.33 ±1.6	33.71 ±0.4	33.13 ±0.4
A3, in water at day-old	30.80 ±0.8	28.52 ±1.0	30.10 ±0.7	32.43 ±0.8	32.24 ±0.8	34.67 ±1.0	35.95 ±0.9	35.76 ±1.1	35.57 ±0.9	32.57 ±0.9	32.87 ±0.3
All Groups	32.47 ±0.5	28.92 ±0.4	31.32 ±0.4	31.95 ±0.4	32.86 ±0.3	35.58 ±0.4	35.98 ±0.4	35.96 ±0.4	36.32 ±0.4	32.98 ±0.4	

Values are Mean ± S.E.

Table C7 (cont). Egg quality shell reflectivity (%) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	32.78 ±0.4	28.78 ±0.3	30.40 ±0.3	31.57 ±0.3	33.43 ±0.4	34.99 ±0.4	35.44 ±0.4	37.01 ±0.4	36.17 ±0.4	33.76 ±0.4	33.44 ±0.1
Control	33.05 ±0.8	30.19 ±0.8	31.52 ±1.0	31.95 ±0.9	34.19 ±1.0	36.10 ±0.9	36.95 ±0.9	37.67 ±1.3	35.48 ±1.1	34.90 ±1.0	34.21 ±0.3
VicS, eyedrop at day-old	32.62 ±1.0	29.14 ±0.8	30.67 ±0.7	31.62 ±0.8	34.10 ±1.0	35.24 ±0.9	35.48 ±0.9	35.95 ±1.0	36.19 ±0.9	34.14 ±1.3	33.51 ±0.3
VicS, coarse spray at day-old	33.57 ±1.2	26.62 ±0.9	29.81 ±0.9	31.9 ±0.9	32.57 ±0.9	34.65 ±1.0	34.67 ±1.1	37.52 ±1.0	36.38 ±1.1	34.62 ±1.2	33.26 ±0.4
VicS, in water at day-old	34.90 ±1.3	29.62 ±1.2	31.71 ±0.8	33.48 ±1.0	34.29 ±1.2	34.24 ±0.7	35.29 ±0.9	36.86 ±1.0	34.57 ±1.0	33.00 ±0.9	33.79 ±0.3
A3, eyedrop at day-old	32.05 ±1.0	28.70 ±1.0	29.52 ±0.9	29.86 ±0.9	32.71 ±0.9	34.14 ±1.0	34.76 ±1.0	37.29 ±1.4	36.90 ±1.0	32.24 ±0.8	32.84 ±0.4
A3, coarse spray at day-old	31.71 ±1.1	28.29 ±0.8	29.57 ±0.8	31.19 ±0.8	33.29 ±1.0	36.00 ±1.2	35.24 ±1.0	36.33 ±0.9	35.38 ±0.8	32.71 ±0.7	32.97 ±0.3
A3, in water at day-old	31.42 ±1.0	28.90 ±0.8	29.95 ±0.9	31.05 ±1.0	32.91 ±0.9	34.24 ±1.0	35.71 ±1.1	37.48 ±1.2	38.29 ±1.3	34.71 ±1.1	33.49 ±0.4
All Groups	32.78 ±0.4	28.78 ±0.3	30.40 ±0.3	31.57 ±0.3	33.43 ±0.4	34.99 ±0.4	35.44 ±0.4	37.01 ±0.4	36.17 ±0.4	33.76 ±0.4	

Values are Mean ± S.E.
Significant difference within initial treatment (<0.0001), ages (<0.0001); Interaction between shed and treatments (0.0002)

Table C8. Egg quality shell breaking strength (Newton) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	41.51 ±0.6	48.20 ±0.5	46.13 ±0.5	45.54 ±0.6	43.95 ±0.7	42.97 ±0.6	41.14 ±0.6	39.52 ±0.7	40.55 ±0.6	35.24 ±0.5	42.49 ±0.2
Control	40.85 ±1.6	46.66 ±1.7	47.77 ±1.0	44.54 ±1.5	43.22 ±2.6	42.56 ±1.9	40.02 ±1.1	41.26 ±1.6	42.32 ±2.4	33.88 ±1.5	42.31 ±0.6
VicS, eyedrop at day-old	41.12 ±1.5	49.86 ±1.0	44.22 ±1.3	43.75 ±1.5	44.51 ±1.4	43.34 ±1.4	38.94 ±1.8	38.95 ±1.2	39.05 ±1.5	34.08 ±1.5	41.82 ±0.5
VicS, coarse spray at day-old	41.49 ±1.9	46.75 ±1.5	46.51 ±1.1	42.66 ±2.3	44.19 ±1.9	41.90 ±2.0	41.72 ±1.5	41.20 ±1.7	39.86 ±1.7	36.97 ±1.2	42.34 ±0.6
VicS, in water at day-old	41.07 ±1.3	47.72 ±1.4	45.28 ±1.5	47.34 ±1.7	44.30 ±1.3	42.95 ±2.1	43.06 ±1.9	39.87 ±1.4	40.65 ±1.2	36.68 ±1.6	42.91 ±0.5
A3, eyedrop at day-old	42.22 ±1.6	49.02 ±1.4	45.91 ±1.2	46.55 ±1.5	42.57 ±2.0	43.30 ±1.1	42.91 ±1.5	36.75 ±1.6	40.13 ±1.4	34.82 ±1.2	42.42 ±0.5
A3, coarse spray at day-old	40.30 ±2.2	47.67 ±1.1	44.95 ±1.1	47.05 ±1.5	45.74 ±1.9	42.13 ±1.3	40.89 ±1.5	36.62 ±2.4	39.23 ±1.7	35.33 ±1.6	42.00 ±0.6
A3, in water at day-old	43.63 ±1.7	49.73 ±1.3	48.24 ±1.6	46.75 ±1.3	43.12 ±1.9	44.62 ±1.5	40.47 ±1.4	42.00 ±2.2	42.61 ±1.6	34.85 ±1.6	43.64 ±0.6
All Groups	41.51 ±0.6	48.20 ±0.5	46.13 ±0.5	45.54 ±0.6	43.95 ±0.7	42.97 ±0.6	41.14 ±0.6	39.52 ±0.7	40.55 ±0.6	35.24 ±0.6	

Values are Mean ± S.E.

Table C8 (cont). Egg quality shell breaking strength (Newtons) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	41.86 ±0.8	45.60 ±0.6	46.02 ±0.6	42.15 ±0.6	42.04 ±0.6	42.56 ±0.5	40.61 ±0.6	38.07 ±0.7	39.38 ±0.6	34.12 ±0.6	41.24 ±0.2
Control	42.47 ±2.3	48.72 ±1.6	47.20 ±1.2	42.47 ±1.9	41.92 ±2.3	44.27 ±1.1	42.22 ±1.5	37.30 ±1.9	39.42 ±1.9	34.36 ±1.2	42.00 ±0.6
VicS, eyedrop at day-old	41.29 ±2.8	46.68 ±1.6	46.20 ±2.5	41.43 ±1.9	43.13 ±1.4	43.75 ±1.2	43.54 ±1.4	41.49 ±1.9	42.25 ±1.3	34.80 ±2.0	42.46 ±0.6
VicS, coarse spray at day-old	42.13 ±2.7	46.50 ±1.4	47.03 ±1.3	43.76 ±1.5	43.33 ±1.3	42.48 ±1.4	41.38 ±1.0	37.80 ±2.2	38.69 ±1.2	31.03 ±1.4	41.41 ±0.6
VicS, in water at day-old	38.66 ±1.8	43.04 ±1.8	43.08 ±1.5	40.07 ±1.8	41.26 ±1.7	41.33 ±1.4	39.34 ±1.6	36.52 ±2.0	39.36 ±1.7	35.11 ±1.5	39.78 ±0.6
A3, eyedrop at day-old	40.65 ±1.6	42.70 ±1.9	45.99 ±1.4	39.63 ±1.5	41.66 ±1.8	39.16 ±1.7	35.85 ±1.8	36.29 ±2.1	39.27 ±1.6	33.53 ±1.9	39.46 ±0.6
A3, coarse spray at day-old	44.34 ±2.0	45.83 ±1.4	46.40 ±1.3	42.53 ±1.5	41.38 ±1.4	42.57 ±1.2	39.13 ±1.4	36.30 ±1.6	37.42 ±1.8	32.38 ±1.7	40.81 ±0.6
A3, in water at day-old	43.79 ±1.8	45.59 ±1.6	46.25 ±1.3	45.17 ±1.4	41.61 ±1.8	44.37 ±2.0	42.80 ±1.4	40.82 ±1.3	39.24 ±1.5	37.63 ±1.2	42.72 ±0.5
All Groups	41.86 ±0.8	45.60 ±0.6	46.02 ±0.6	42.15 ±0.6	42.04 ±0.6	42.56 ±0.6	40.61 ±0.6	38.08 ±0.7	39.38 ±0.6	34.12 ±0.6	

Values are Mean ± S.E.

Significant difference within shed (<0.0001), initial treatment (0.0007), ages (<0.0001); Interaction between shed and treatments (0.0021)

Table C9. Egg quality shell deformation (mm) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	280.63 ±4.6	277.41 ±4.9	276.77 ±5.8	263.56 ±5.2	263.61 ±5.7	271.16 ±7.0	254.35 ±6.5	261.29 ±7.4	280.41 ±8.2	233.31 ±6.7	266.24 ±2.0
Control	272.38 ±6.8	266.19 ±9.9	273.81 ±7.9	277.14 ±25.1	283.81 ±17.5	268.10 ±13.8	251.43 ±12.5	251.90 ±14.6	295.24 ±22.8	213.81 ±6.3	265.38 ±4.9
VicS, eyedrop at day-old	273.00 ±8.6	275.24 ±9.4	273.10 ±25.7	270.00 ±17.6	256.67 ±11.5	259.05 ±12.4	247.14 ±18.42	256.67 ±20.6	252.86 ±14.4	218.50 ±6.5	258.34 ±5.0
VicS, coarse spray at day-old	303.33 ±21.6	269.05 ±6.6	272.86 ±6.5	248.50 ±8.5	263.81 ±15.8	277.62 ±24.8	262.86 ±20.6	294.76 ±25.7	320.00 ±35.3	244.29 ±17.7	275.63 ±6.5
VicS, in water at day-old	278.57 ±8.8	275.71 ±9.8	278.57 ±12.9	259.52 ±9.6	262.33 ±12.4	289.05 ±19.34	278.09 ±31.4	236.19 ±8.6	278.57 ±18.5	220.48 ±10.1	265.71 ±5.1
A3, eyedrop at day-old	283.16 ±6.5	271.90 ±10.1	275.71 ±14.9	264.76 ±5.5	263.33 ±22.2	290.95 ±29.38	256.19 ±8.3	216.19 ±6.7	249.05 ±9.5	241.43 ±27.7	261.06 ±5.3
A3, coarse spray at day-old	272.50 ±13.6	306.19 ±26.1	287.62 ±21.9	257.62 ±6.6	274.29 ±12.4	262.38 ±10.6	243.81 ±7.2	297.14 ±27.4	275.24 ±18.9	236.67 ±7.2	271.34 ±5.4
A3, in water at day-old	281.00 ±11.6	277.62 ±6.8	275.71 ±8.3	266.67 ±13.1	240.95 ±10.1	250.95 ±9.9	240.95 ±8.8	276.19 ±18.9	293.81 ±23.1	258.50 ±30.3	266.20 ±5.0
All Groups	280.63 ±4.6	277.41 ±4.9	276.77 ±5.8	263.56 ±5.2	263.60 ±5.7	271.16 ±7.0	254.35 ±6.5	261.29 ±7.4	280.41 ±8.2	233.31 ±6.7	

Values are Mean ± S.E.

Table C9 (cont). Egg quality shell deformation (mm) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	303.08 ±5.8	274.93 ±4.4	285.51 ±6.3	262.38 ±7.0	281.70 ±7.3	281.09 ±8.6	258.78 ±5.8	254.01 ±6.8	260.20 ±6.9	244.69 ±7.1	270.55 ±2.2
Control	326.00 ±18.0	286.19 ±12.6	290.48 ±21.0	238.57 ±10.2	306.19 ±31.42	342.38 ±35.8	284.76 ±19.3	261.43 ±17.3	268.10 ±21.0	268.09 ±29.3	287.03 ±7.4
VicS, eyedrop at day-old	299.52 ±14.2	274.76 ±9.3	270.95 ±10.0	299.05 ±32.0	272.86 ±18.9	266.19 ±14.0	254.29 ±6.6	272.38 ±16.9	245.71 ±5.0	260.48 ±19.4	271.62 ±5.2
VicS, coarse spray at day-old	300.95 ±9.6	269.05 ±6.9	298.10 ±15.0	235.71 ±16.6	267.14 ±6.8	308.10 ±29.6	270.95 ±20.52	260.95 ±22.3	238.57 ±8.3	230.00 ±17.9	267.95 ±5.6
VicS, in water at day-old	270.95 ±8.5	273.33 ±6.8	292.86 ±18.9	256.19 ±12.1	276.19 ±16.78	262.9 ±16.5	247.62 ±9.7	229.52 ±12.0	292.86 ±29.9	242.86 ±15.3	264.52 ±5.1
A3, eyedrop at day-old	332.86 ±26.5	253.00 ±11.31	260.00 ±8.7	262.38 ±16.2	265.71 ±16.16	231.43 ±10.2	250.48 ±20.2	239.52 ±17.2	293.33 ±24.3	226.67 ±14.7	261.58 ±5.8
A3, coarse spray at day-old	298.00 ±9.7	287.14 ±14.7	277.14 ±13.1	260.48 ±9.5	273.33 ±14.41	291.90 ±22.50	233.33 ±12.0	265.24 ±25.6	247.62 ±10.4	230.48 ±17.9	266.32 ±5.2
A3, in water at day-old	293.16 ±8.4	280.00 ±16.6	309.05 ±24.3	284.29 ±20.9	310.48 ±22.6	264.76 ±12.9	270.00 ±12.0	249.05 ±12.3	235.24 ±10.6	254.29 ±12.7	274.86 ±5.3
All Groups	303.08 ±5.8	274.93 ±4.4	285.51 ±6.3	262.38 ±7.0	281.70 ±7.3	281.09 ±8.6	258.78 ±5.8	254.01 ±6.8	260.20 ±6.9	244.69 ±7.1	

Values are Mean ± S.E.
Significant difference within ages across not revaccinated and revaccinated (<0.0001); Interaction between shed and ages (0.0461)

Table C10. Egg quality shell weight (g) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	4.68 ±0.1	5.89 ±0.05	6.14 ±0.04	6.04 ±0.05	6.45 ±0.05	6.29 ±0.04	6.24 ±0.04	6.26 ±0.04	6.26 ±0.05	6.04 ±0.1	6.03 ±0.02
Control	4.57 ±0.1	5.82 ±0.1	6.16 ±0.1	5.95 ±0.1	6.27 ±0.2	6.12 ±0.1	6.09 ±0.1	6.33 ±0.1	6.23 ±0.2	6.00 ±0.1	5.96 ±0.05
VicS, eyedrop at day-old	4.74 ±0.1	5.96 ±0.1	6.30 ±0.1	6.08 ±0.2	6.50 ±0.1	6.32 ±0.1	6.16 ±0.2	6.20 ±0.1	6.23 ±0.1	5.69 ±0.3	6.03 ±0.06
VicS, coarse spray at day-old	4.78 ±0.1	5.84 ±0.1	6.10 ±0.1	5.83 ±0.2	6.40 ±0.1	6.14 ±0.1	6.32 ±0.1	6.44 ±0.1	6.11 ±0.2	6.21 ±0.1	6.02 ±0.05
VicS, in water at day-old	4.67 ±0.1	6.04 ±0.1	6.03 ±0.1	6.16 ±0.1	6.58 ±0.1	6.42 ±0.1	6.37 ±0.1	6.22 ±0.1	6.24 ±0.1	6.20 ±0.1	6.09 ±0.05
A3, eyedrop at day-old	4.79 ±0.1	5.99 ±0.1	6.09 ±0.1	6.01 ±0.1	6.44 ±0.1	6.20 ±0.1	6.30 ±0.1	6.24 ±0.1	6.35 ±0.1	6.13 ±0.1	6.07 ±0.05
A3, coarse spray at day-old	4.64 ±0.2	5.61 ±0.1	6.08 ±0.1	6.23 ±0.1	6.47 ±0.1	6.45 ±0.1	6.25 ±0.1	6.07 ±0.1	6.29 ±0.1	6.00 ±0.2	6.01 ±0.06
A3, in water at day-old	4.62 ±0.1	5.98 ±0.1	6.20 ±0.1	6.00 ±0.1	6.48 ±0.1	6.34 ±0.1	6.21 ±0.1	6.32 ±0.1	6.37 ±0.1	6.08 ±0.2	6.07 ±0.05
All Groups	4.68 ±0.05	5.89 ±0.05	6.14 ±0.04	6.04 ±0.05	6.45 ±0.05	6.29 ±0.04	6.24 ±0.04	6.26 ±0.04	6.26 ±0.05	6.05 ±0.1	

Values are Mean ± S.E.

Table C10 (cont). Egg quality shell weight (g) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	4.74 ±0.1	5.72 ±0.06	6.23 ±0.05	6.22 ±0.04	6.33 ±0.04	6.28 ±0.05	6.28 ±0.04	6.20 ±0.05	6.09 ±0.05	5.99 ±0.1	6.01 ±0.02
Control	4.54 ±0.1	5.76 ±0.1	6.11 ±0.1	6.23 ±0.1	6.21 ±0.1	6.12 ±0.2	6.25 ±0.1	5.91 ±0.2	5.81 ±0.2	5.91 ±0.1	5.89 ±0.06
VicS, eyedrop at day-old	4.75 ±0.1	5.93 ±0.1	6.12 ±0.1	6.26 ±0.1	6.23 ±0.1	6.29 ±0.1	6.24 ±0.1	6.39 ±0.1	6.31 ±0.1	5.99 ±0.2	6.05 ±0.05
VicS, coarse spray at day-old	4.71 ±0.1	5.72 ±0.1	6.31 ±0.1	6.47 ±0.1	6.47 ±0.1	6.30 ±0.1	6.22 ±0.1	6.19 ±0.1	6.02 ±0.1	5.92 ±0.2	6.03 ±0.05
VicS, in water at day-old	4.67 ±0.1	5.53 ±0.1	6.14 ±0.2	5.97 ±0.1	6.33 ±0.2	6.16 ±0.1	6.20 ±0.1	6.24 ±0.1	6.14 ±0.1	6.00 ±0.1	5.93 ±0.05
A3, eyedrop at day-old	4.68 ±0.1	5.55 ±0.3	6.26 ±0.1	6.10 ±0.1	6.40 ±0.1	6.19 ±0.1	6.24 ±0.1	6.14 ±0.2	6.19 ±0.1	6.10 ±0.1	5.99 ±0.06
A3, coarse spray at day-old	4.69 ±0.1	5.85 ±0.1	6.30 ±0.1	6.22 ±0.1	6.30 ±0.1	6.36 ±0.1	6.32 ±0.1	6.19 ±0.1	6.05 ±0.1	6.02 ±0.2	6.03 ±0.05
A3, in water at day-old	5.18 ±0.1	5.73 ±0.1	6.38 ±0.1	6.31 ±0.1	6.39 ±0.1	6.57 ±0.1	6.5 ±0.1	6.36 ±0.1	6.11 ±0.2	5.99 ±0.2	6.16 ±0.05
All Groups	4.74 ±0.05	5.73 ±0.06	6.23 ±0.05	6.22 ±0.04	6.33 ±0.04	6.29 ±0.05	6.28 ±0.04	6.20 ±0.05	6.09 ±0.05	5.99 ±0.06	

Values are Mean ± S.E.
Significant difference within initial treatment (0.0027), ages (<0.0001); Interaction between shed and ages (0.0034)

Table C11. Egg quality percentage of egg shell (%) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	10.03 ±0.1	10.87 ±0.1	10.50 ±0.1	10.45 ±0.1	10.42 ±0.1	10.18 ±0.1	9.93 ±0.1	9.86 ±0.1	10.01 ±0.1	9.55 ±0.1	10.18 ±0.03
Control	9.98 ±0.2	10.88 ±0.1	10.68 ±0.1	10.53 ±0.1	10.12 ±0.3	10.07 ±0.2	9.97 ±0.2	10.19 ±0.2	10.04 ±0.2	9.71 ±0.2	10.19 ±0.1
VicS, eyedrop at day-old	10.10 ±0.1	10.82 ±0.2	10.34 ±0.2	10.49 ±0.2	10.50 ±0.1	10.24 ±0.1	9.81 ±0.2	9.78 ±0.2	9.81 ±0.1	8.87 ±0.4	10.08 ±0.1
VicS, coarse spray at day-old	10.05 ±0.2	10.72 ±0.1	10.45 ±0.1	10.32 ±0.3	10.48 ±0.1	10.08 ±0.1	10.10 ±0.1	9.90 ±0.2	9.78 ±0.3	9.69 ±0.1	10.16 ±0.1
VicS, in water at day-old	9.9 ±0.3	10.94 ±0.1	10.32 ±0.2	10.53 ±0.2	10.51 ±0.1	10.19 ±0.1	10.15 ±0.1	9.91 ±0.2	10.03 ±0.1	9.79 ±0.1	10.23 ±0.1
A3, eyedrop at day-old	10.39 ±0.2	11.13 ±0.2	10.60 ±0.1	10.21 ±0.2	10.42 ±0.1	10.16 ±0.1	9.91 ±0.1	9.77 ±0.1	10.02 ±0.2	9.55 ±0.1	10.22 ±0.1
A3, coarse spray at day-old	9.60 ±0.4	10.51 ±0.3	10.36 ±0.1	10.35 ±0.1	10.29 ±0.1	10.09 ±0.1	9.73 ±0.2	9.43 ±0.2	9.95 ±0.2	9.60 ±0.2	9.99 ±0.1
A3, in water at day-old	10.25 ±0.2	11.10 ±0.2	10.77 ±0.1	10.71 ±0.2	10.62 ±0.1	10.41 ±0.2	10.10 ±0.2	10.08 ±0.1	10.42 ±0.1	9.62 ±0.3	10.41 ±0.1
All Groups	10.03 ±0.1	10.87 ±0.1	10.50 ±0.1	10.45 ±0.1	10.42 ±0.1	10.18 ±0.1	9.93 ±0.1	9.87 ±0.1	10.01 ±0.1	9.55 ±0.1	

Values are Mean ± S.E.
Significant difference within shed (<0.0001), ages (<0.0001); interaction between shed and initial treatment (0.0002)

Table C11 (cont). Egg quality percentage of egg shell (%) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	9.95 ±0.1	10.50 ±0.1	10.48 ±0.1	10.09 ±0.1	10.12 ±0.1	9.97 ±0.1	9.83 ±0.1	9.67 ±0.1	9.71 ±0.1	9.42 ±0.1	9.98 0.02
Control	9.83 ±0.3	10.81 ±0.1	10.58 ±0.1	10.37 ±0.2	10.19 ±0.2	9.82 ±0.2	9.75 ±0.2	9.50 ±0.3	9.63 ±0.3	9.60 ±0.2	10.01 0.1
VicS, eyedrop at day-old	10.04 ±0.3	10.53 ±0.2	10.45 ±0.2	10.21 ±0.2	10.11 ±0.1	10.05 ±0.1	9.82 ±0.1	9.99 ±0.2	9.93 ±0.1	9.52 ±0.2	10.07 0.1
VicS, coarse spray at day-old	9.80 ±0.3	10.74 ±0.1	10.44 ±0.1	10.26 ±0.2	10.29 ±0.2	9.97 ±0.1	9.80 ±0.1	9.71 ±0.2	9.64 ±0.2	9.11 ±0.2	9.98 0.1
VicS, in water at day-old	9.64 ±0.2	10.37 ±0.2	10.27 ±0.2	9.94 ±0.1	9.98 ±0.2	9.94 ±0.1	9.64 ±0.1	9.66 ±0.2	9.83 ±0.2	9.62 ±0.2	9.89 0.1
A3, eyedrop at day-old	10.00 ±0.2	9.96 ±0.4	10.42 ±0.1	9.78 ±0.1	10.16 ±0.2	9.85 ±0.1	9.80 ±0.2	9.40 ±0.2	9.74 ±0.1	9.37 ±0.1	9.85 0.1
A3, coarse spray at day-old	10.25 ±0.2	10.57 ±0.3	10.65 ±0.1	10.04 ±0.1	10.03 ±0.2	9.91 ±0.1	9.97 ±0.2	9.79 ±0.1	9.65 ±0.2	9.54 ±0.3	10.04 0.1
A3, in water at day-old	10.11 ±0.2	10.50 ±0.2	10.52 ±0.1	10.05 ±0.1	10.04 ±0.2	10.24 ±0.1	10.05 ±0.2	9.64 ±0.1	9.58 ±0.2	9.21 ±0.3	10.00 0.1
All Groups	9.95 0.1	10.50 0.1	10.48 0.1	10.09 0.1	10.12 0.1	9.97 0.05	9.83 0.06	9.67 0.1	9.72 0.1	9.43 0.1	

Values are Mean ± S.E.

Table C12. Egg quality shell thickness (μm) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	402.84 ±3.0	446.23 ±2.1	441.63 ±2.1	439.85 ±3.4	441.64 ±2.3	439.00 ±2.0	436.72 ±2.1	435.63 ±2.4	441.33 ±2.8	427.67 ±3.5	435.34 ±0.9
Control	398.48 ±8.6	446.95 ±3.7	448.76 ±4.5	436.38 ±5.2	434.24 ±9.6	432.81 ±4.4	429.24 ±5.2	439.48 ±4.4	447.52 ±7.9	434.76 ±6.9	434.86 ±2.2
VicS, eyedrop at day-old	400.05 ±4.2	443.67 ±5.2	437.48 ±6.9	440.52 ±6.9	448.38 ±5.9	434.14 ±6.0	431.95 ±8.1	431.81 ±7.1	432.19 ±6.2	403.14 ±16.5	430.48 ±2.7
VicS, coarse spray at day-old	406.62 ±6.4	435.62 ±6.1	436.09 ±4.1	419.62 ±12.8	436.81 ±4.7	428.62 ±5.4	441.81 ±5.0	439.57 ±6.1	435.24 ±11.5	432.95 ±7.2	431.30 ±2.4
VicS, in water at day-old	411.76 ±5.1	442.33 ±4.6	437.43 ±6.0	446.90 ±10.0	444.86 ±5.2	442.00 ±4.6	444.90 ±3.9	437.95 ±8.4	434.48 ±5.0	433.38 ±6.4	437.60 ±2.0
A3, eyedrop at day-old	420.16 ±7.2	458.05 ±7.0	443.57 ±4.1	432.19 ±7.2	444.24 ±3.6	446.57 ±5.4	440.14 ±6.1	436.48 ±5.3	438.57 ±4.1	431.81 ±6.3	439.36 ±1.9
A3, coarse spray at day-old	385.71 ±12.6	446.62 ±5.8	435.38 ±6.8	457.71 ±10.6	433.33 ±5.5	443.43 ±4.6	429.52 ±4.3	425.05 ±6.5	443.90 ±8.4	427.05 ±8.6	432.77 ±2.7
A3, in water at day-old	398.40 ±5.9	450.38 ±5.7	452.67 ±5.9	445.62 ±7.4	449.62 ±5.7	445.43 ±5.9	439.48 ±5.8	439.09 ±5.2	457.38 ±5.2	430.57 ±9.0	441.07 ±2.2
All Groups	402.84 ±3.0	446.23 ±2.1	441.63 ±2.1	439.85 ±3.4	441.64 ±2.3	439.00 ±2.0	436.72 ±2.1	435.63 ±2.4	441.33 ±2.8	427.67 ±3.6	

Values are Mean ± S.E.

Table C12 (cont). Egg quality shell thickness (µm) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	402.51 ±3.0	437.68 ±3.1	444.37 ±2.4	433.59 ±2.3	428.54 ±2.2	435.05 ±2.2	435.58 ±2.2	430.29 ±2.5	430.78 ±2.8	425.37 ±2.8	430.43 ±0.9
Control	397.95 ±8.9	451.05 ±5.4	448.71 ±6.2	442.90 ±6.9	432.19 ±6.2	423.05 ±8.6	437.67 ±5.3	422.09 ±9.1	422.86 ±10.9	426.38 ±7.2	430.64 ±2.6
VicS, eyedrop at day-old	405.09 ±9.2	440.81 ±5.8	437.81 ±8.6	437.86 ±5.7	426.19 ±5.1	429.05 ±5.1	430.05 ±5.1	439.24 ±5.5	442.48 ±6.1	430.29 ±7.4	431.89 ±2.1
VicS, coarse spray at day-old	403.19 ±10.4	442.43 ±5.2	443.05 ±5.6	440.33 ±6.6	431.90 ±5.1	435.43 ±4.6	433.86 ±5.2	431.57 ±5.3	427.52 ±7.1	414.24 ±6.5	430.35 ±2.1
VicS, in water at day-old	395.95 ±8.1	430.52 ±6.4	438.52 ±6.0	425.05 ±4.6	431.48 ±5.8	433.52 ±6.1	430.71 ±6.9	431.43 ±7.7	435.71 ±6.3	428.62 ±7.1	428.15 ±2.1
A3, eyedrop at day-old	400.38 ±8.1	418.67 ±15.9	442.67 ±6.8	418.19 ±5.4	421.29 ±5.5	432.86 ±3.9	431.00 ±5.6	422.43 ±7.8	425.62 ±4.1	419.62 ±4.2	423.27 ±2.4
A3, coarse spray at day-old	405.52 ±6.6	439.00 ±6.5	454.24 ±6.2	433.14 ±4.9	427.62 ±5.8	440.76 ±5.9	439.90 ±5.7	436.57 ±5.6	430.81 ±7.4	426.57 ±11.3	433.41 ±2.3
A3, in water at day-old	410.00 ±4.8	441.29 ±5.7	445.62 ±5.7	437.67 ±6.2	429.1 ±7.0	450.71 ±5.1	445.86 ±6.7	428.67 ±4.6	430.43 ±8.0	431.90 ±8.7	435.37 ±2.1
All Groups	402.51 ±3.0	437.68 ±3.1	444.37 ±2.4	433.59 ±2.3	428.54 ±2.2	435.05 ±2.3	435.58 ±2.2	430.28 ±2.5	430.78 ±2.8	425.37 ±2.9	

Values are Mean ± S.E.
Significant difference within shed (<0.0001), initial treatment (0.0175), ages (<0.0001); Interaction between shed and treatments (0.0004)

Table C13. Egg quality albumen height (mm) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	7.97 ±0.1	8.11 ±0.1	7.35 ±0.1	7.22 ±0.1	7.05 ±0.1	6.44 ±0.1	6.94 ±0.1	7.53 ±0.1	7.67 ±0.1	7.52 ±0.1	7.38 ±0.03
Control	8.51 ±0.2	8.10 ±0.2	7.10 ±0.2	7.51 ±0.2	7.18 ±0.2	6.70 ±0.2	7.40 ±0.2	8.38 ±0.2	8.22 ±0.3	7.54 ±0.3	7.66 ±0.1
VicS, eyedrop at day-old	8.31 ±0.3	8.33 ±0.2	7.05 ±0.2	7.35 ±0.3	7.23 ±0.2	6.40 ±0.3	6.83 ±0.2	7.33 ±0.3	8.08 ±0.3	7.61 ±0.3	7.45 ±0.1
VicS, coarse spray at day-old	8.13 ±0.2	8.29 ±0.2	7.72 ±0.2	7.41 ±0.2	7.00 ±0.3	6.54 ±0.2	6.71 ±0.3	7.36 ±0.3	7.12 ±0.4	7.24 ±0.3	7.35 ±0.1
VicS, in water at day-old	8.02 ±0.2	8.14 ±0.2	7.50 ±0.2	7.09 ±0.2	7.02 ±0.2	6.56 ±0.2	6.88 ±0.3	7.42 ±0.3	7.64 ±0.2	7.55 ±0.3	7.38 ±0.1
A3, eyedrop at day-old	8.26 ±0.2	8.16 ±0.2	7.63 ±0.2	7.57 ±0.3	7.31 ±0.2	6.37 ±0.3	7.05 ±0.3	8.06 ±0.3	7.79 ±0.2	7.87 ±0.3	7.60 ±0.1
A3, coarse spray at day-old	7.53 ±0.4	8.06 ±0.2	7.32 ±0.2	6.90 ±0.3	7.08 ±0.2	6.50 ±0.2	7.03 ±0.2	7.36 ±0.3	7.85 ±0.3	7.87 ±0.2	7.35 ±0.1
A3, in water at day-old	7.04 ±0.2	7.71 ±0.2	7.13 ±0.2	6.74 ±0.2	6.50 ±0.3	5.99 ±0.2	6.65 ±0.3	6.81 ±0.2	7.01 ±0.3	6.97 ±0.4	6.85 ±0.1
All Groups	7.97 ±0.1	8.11 ±0.1	7.35 ±0.1	7.23 ±0.1	7.05 ±0.1	6.44 ±0.1	6.94 ±0.1	7.53 ±0.1	7.68 ±0.1	7.52 ±0.1	

Values are Mean ± S.E.

Table C13 (cont). Egg quality albumen height (mm) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	80.02 ±0.1	8.16 ±0.1	7.25 ±0.1	7.19 ±0.1	6.93 ±0.1	6.45 ±0.1	6.83 ±0.1	7.36 ±0.1	7.68 ±0.1	7.52 ±0.1	7.34 ±0.04
Control	8.45 ±0.3	8.59 ±0.2	7.00 ±0.2	7.27 ±0.3	7.26 ±0.3	7.22 ±0.3	7.17 ±0.3	7.49 ±0.3	8.53 ±0.4	7.47 ±0.3	7.64 ±0.1
VicS, eyedrop at day-old	8.74 ±0.2	8.85 ±0.2	7.32 ±0.3	7.36 ±0.2	7.15 ±0.2	6.70 ±0.3	6.83 ±0.3	7.49 ±0.2	8.22 ±0.3	7.31 ±0.3	7.60 ±0.1
VicS, coarse spray at day-old	8.55 ±0.2	8.49 ±0.2	7.45 ±0.2	7.34 ±0.2	7.43 ±0.2	6.59 ±0.3	6.99 ±0.2	7.40 ±0.2	7.84 ±0.2	7.69 ±0.3	7.58 ±0.1
VicS, in water at day-old	8.04 ±0.2	8.05 ±0.2	7.22 ±0.2	7.03 ±0.3	6.98 ±0.3	6.60 ±0.3	6.81 ±0.3	7.17 ±0.4	7.06 ±0.4	7.07 ±0.4	7.20 ±0.1
A3, eyedrop at day-old	7.96 ±0.2	7.99 ±0.3	7.25 ±0.2	7.06 ±0.2	6.54 ±0.2	6.05 ±0.3	6.61 ±0.3	7.51 ±0.2	7.26 ±0.3	7.88 ±0.3	7.21 ±0.1
A3, coarse spray at day-old	7.16 ±0.3	7.22 ±0.3	7.39 ±0.2	7.19 ±0.2	6.62 ±0.2	5.86 ±0.2	7.01 ±0.2	7.32 ±0.2	7.53 ±0.2	7.72 ±0.2	7.10 ±0.1
A3, in water at day-old	7.16 ±0.3	7.92 ±0.3	7.23 ±0.2	7.09 ±0.2	6.52 ±0.2	6.11 ±0.2	6.39 ±0.2	7.13 ±0.2	7.34 ±0.3	7.50 ±0.3	7.03 ±0.1
All Groups	8.02 ±0.1	8.16 ±0.1	7.25 ±0.1	7.19 ±0.1	6.93 ±0.1	6.45 ±0.1	6.83 ±0.1	7.36 ±0.1	7.68 ±0.1	7.52 ±0.1	

Values are Mean ± S.E.

Significant difference within initial treatment (<0.0001), ages (<0.0001); Interaction between shed and treatments (0.0004), between treatment and ages (0.0020)

Table C14. Egg quality Haugh unit (HU) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	92.38 ±0.6	91.06 ±0.5	85.84 ±0.5	84.94 ±0.6	82.69 ±0.5	78.33 ±0.7	85.20 ±0.2	84.96 ±0.7	85.93 ±0.7	84.78 ±0.8	85.20 ±0.2
Control	95.71 ±0.8	91.43 ±0.9	84.57 ±1.0	87.19 ±1.3	83.67 ±1.3	80.52 ±1.6	84.71 ±1.6	90.43 ±1.2	89.38 ±1.8	85.09 ±2.3	87.27 ±0.5
VicS, eyedrop at day-old	94.15 ±1.3	92.19 ±1.1	84.10 ±1.2	85.76 ±1.5	84.10 ±1.4	77.76 ±2.3	80.86 ±1.8	83.62 ±2.0	88.00 ±2.0	85.14 ±1.8	85.53 ±0.6
VicS, coarse spray at day-old	93.14 ±1.3	92.05 ±1.0	88.05 ±1.0	86.67 ±1.2	82.38 ±1.7	79.48 ±1.7	79.71 ±2.1	83.29 ±2.1	81.43 ±3.1	82.86 ±2.0	84.91 ±0.7
VicS, in water at day-old	92.62 ±1.2	95.95 ±1.5	86.57 ±1.4	84.00 ±1.4	82.33 ±1.4	78.95 ±1.6	80.52 ±2.5	84.81 ±1.4	86.19 ±1.7	85.00 ±2.1	85.20 ±0.6
A3, eyedrop at day-old	94.10 ±1.2	91.76 ±1.2	87.67 ±1.2	86.67 ±1.4	84.43 ±1.3	77.76 ±2.4	81.91 ±1.8	88.14 ±1.5	86.86 ±1.4	86.86 ±1.6	86.54 ±0.6
A3, coarse spray at day-old	89.00 ±2.2	90.81 ±1.6	85.38 ±1.3	81.95 ±1.9	82.81 ±1.3	78.19 ±1.8	81.95 ±1.3	83.43 ±2.2	87.10 ±1.6	87.86 ±1.7	84.85 ±0.6
A3, in water at day-old	87.95 ±1.4	88.24 ±1.8	84.52 ±1.3	82.33 ±1.5	79.10 ±1.8	75.62 ±1.5	79.48 ±2.2	81.00 ±1.3	82.57 ±1.7	80.62 ±2.8	82.12 ±0.6
All Groups	92.38 ±0.6	91.06 ±0.5	85.84 ±0.5	84.94 ±0.6	82.69 ±0.6	78.33 ±0.7	81.31 ±0.7	84.96 ±0.7	85.93 ±0.8	84.78 ±0.8	

Values are Mean ± S.E.

Significant difference within shed (0.0237), initial treatment (<0.0001), age (<0.0001); interaction between shed and initial treatment (0.0020)

Table C14 (cont). Egg quality Haugh unit (HU) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	92.22 ±0.7	91.16 ±0.6	6.70 ±0.6	83.70 ±0.6	81.51 ±0.7	77.80 ±0.8	84.53 ±0.3	83.63 ±0.8	85.82 ±0.8	84.47 ±0.9	84.53 ±0.3
Control	94.10 ±2.8	93.95 ±1.2	83.81 ±1.2	84.24 ±2.1	83.95 ±2.1	83.38 ±1.9	82.43 ±2.1	85.00 ±2.0	91.29 ±2.0	85.09 ±2.0	86.69 ±0.7
VicS, eyedrop at day-old	96.29 ±1.0	94.19 ±1.5	85.10 ±1.8	84.86 ±1.5	83.43 ±1.3	79.48 ±2.4	79.52 ±2.9	84.86 ±1.6	88.86 ±0.9	83.48 ±2.2	86.00 ±0.7
VicS, coarse spray at day-old	94.95 ±1.4	93.71 ±0.9	87.24 ±2.0	85.05 ±1.4	84.71 ±1.6	78.48 ±2.4	81.71 ±1.7	84.19 ±1.9	87.19 ±1.6	84.05 ±3.7	86.13 ±0.7
VicS, in water at day-old	92.62 ±0.9	91.05 ±1.3	84.38 ±1.3	82.81 ±2.0	81.57 ±1.7	79.33 ±2.1	80.00 ±2.1	80.90 ±3.5	81.19 ±2.9	81.67 ±2.6	83.55 ±0.7
A3, eyedrop at day-old	92.52 ±1.1	89.67 ±1.9	84.76 ±1.1	82.76 ±1.3	78.67 ±1.9	74.90 ±2.1	78.62 ±2.1	84.48 ±1.6	83.14 ±2.3	86.10 ±2.7	83.56 ±0.7
A3, coarse spray at day-old	88.33 ±1.7	85.05 ±2.5	85.43 ±1.6	83.43 ±1.6	79.48 ±1.6	73.67 ±1.3	81.91 ±1.6	84.14 ±1.1	85.48 ±1.3	86.57 ±1.2	83.35 ±0.6
A3, in water at day-old	86.26 ±2.0	90.48 ±1.2	83.81 ±1.0	82.86 ±1.3	78.76 ±1.3	75.33 ±1.6	77.48 ±1.7	81.86 ±1.6	83.57 ±1.3	84.33 ±1.9	82.44 ±0.6
All Groups	92.22 ±0.7	91.16 ±0.6	84.93 ±0.6	83.71 ±0.6	81.51 ±0.7	77.80 ±0.8	80.24 ±0.8	83.63 ±0.8	85.82 ±0.8	84.47 ±0.9	

Values are Mean ± S.E.

Significant difference within shed (0.0237), within initial treatment (<0.0001), ages (<0.0001); Interaction between shed and treatments (0.0020)

Table C15. Egg quality yolk colour (Roche scale) of ISA brown hens following IB revaccination in different IB vaccination protocols in laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Not revaccinated	9.58 ±0.1	11.31 ±0.1	11.41 ±0.1	11.19 ±0.1	11.91 ±0.1	11.71 ±0.1	12.01 ±0.1	11.55 ±0.1	10.51 ±0.1	10.98 ±0.1	11.22 ±0.03
Control	9.57 ±0.2	11.19 ±0.2	11.62 ±0.2	11.29 ±0.2	12.05 ±0.2	11.81 ±0.2	12.05 ±0.1	11.62 ±0.1	10.62 ±0.2	10.67 ±0.2	11.25 ±0.1
VicS, eyedrop at day-old	9.45 ±0.2	11.48 ±0.2	11.52 ±0.1	11.48 ±0.2	11.71 ±0.2	11.76 ±0.1	12.00 ±0.1	11.38 ±0.2	10.76 ±0.1	11.14 ±0.2	11.28 ±0.1
VicS, coarse spray at day-old	9.81 ±0.2	11.24 ±0.1	11.48 ±0.1	11.43 ±0.2	11.91 ±0.1	11.81 ±0.1	12.38 ±0.1	11.33 ±0.2	10.57 ±0.2	10.90 ±0.1	11.29 ±0.1
VicS, in water at day-old	9.60 ±0.1	11.24 ±0.2	11.19 ±0.2	11.33 ±0.2	12.05 ±0.1	11.71 ±0.2	12.14 ±0.2	11.76 ±0.1	10.29 ±0.1	11.09 ±0.2	11.25 ±0.1
A3, eyedrop at day-old	9.47 ±0.2	11.43 ±0.2	11.38 ±0.1	11.10 ±0.2	12.00 ±0.1	11.67 ±0.1	11.95 ±0.2	11.76 ±0.1	10.62 ±0.2	11.09 ±0.1	11.26 ±0.1
A3, coarse spray at day-old	9.71 ±0.2	11.24 ±0.2	11.29 ±0.2	10.86 ±0.2	11.95 ±0.2	11.76 ±0.1	11.91 ±0.2	11.67 ±0.1	10.76 ±0.2	10.90 ±0.1	11.21 ±0.1
A3, in water at day-old	9.40 ±0.2	11.33 ±0.1	11.43 ±0.1	10.86 ±0.2	11.67 ±0.2	11.48 ±0.3	11.67 ±0.2	11.33 ±0.2	9.95 ±0.2	11.05 ±0.2	11.02 ±0.1
All Groups	9.58 ±0.1	11.31 ±0.1	11.41 ±0.06	11.19 ±0.1	11.91 ±0.06	11.71 ±0.06	12.01 ±0.06	11.55 ±0.06	10.51 ±0.1	10.98 ±0.06	

Values are Mean ± S.E.

Table C15 (cont). Egg quality yolk color (Roche scale) of ISA brown hens following IB revaccination in different IB vaccination protocols and revaccination every 8 weeks during the laying phase

	Wk 20	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52	Wk 56	All Ages
Revaccinated	9.76 ±0.1	11.09 ±0.1	11.98 ±0.1	11.27 ±0.1	11.76 ±0.1	11.64 ±0.1	11.95 ±0.1	11.33 ±0.1	10.88 ±0.1	10.65 ±0.1	11.24 ±0.03
Control	9.40 ±0.2	10.91 ±0.2	11.71 ±0.2	11.14 ±0.2	11.71 ±0.2	11.71 ±0.2	12.09 ±0.1	11.43 ±0.1	10.90 ±0.2	10.57 ±0.2	11.17 ±0.1
VicS, eyedrop at day-old	9.90 ±0.2	10.62 ±0.2	12.10 ±0.1	11.33 ±0.2	11.86 ±0.2	11.57 ±0.2	11.95 ±0.1	11.43 ±0.1	10.90 ±0.2	10.57 ±0.2	11.22 ±0.1
VicS, coarse spray at day-old	9.52 ±0.2	11.10 ±0.2	12.29 ±0.1	11.14 ±0.2	11.71 ±0.2	11.52 ±0.2	11.90 ±0.1	11.19 ±0.1	10.81 ±0.2	10.71 ±0.2	11.19 ±0.1
VicS, in water at day-old	9.76 ±0.2	11.10 ±0.2	12.14 ±0.2	11.10 ±0.2	11.76 ±0.2	11.62 ±0.2	11.81 ±0.2	11.48 ±0.1	10.95 ±0.2	10.38 ±0.2	11.21 ±0.1
A3, eyedrop at day-old	9.95 ±0.2	11.33 ±0.1	11.86 ±0.1	66.67 ±0.2	11.76 ±0.1	11.71 ±0.1	12.14 ±0.2	11.48 ±0.2	11.05 ±0.2	10.90 ±0.2	11.39 ±0.1
A3, coarse spray at day-old	9.81 ±0.1	11.38 ±0.2	11.62 ±0.2	11.29 ±0.2	11.81 ±0.2	11.67 ±0.2	11.81 ±0.1	11.24 ±0.1	10.62 ±0.2	10.81 ±0.2	11.21 ±0.1
A3, in water at day-old	10.00 ±0.2	11.24 ±0.2	12.14 ±0.2	11.24 ±0.2	11.71 ±0.2	11.67 ±0.2	11.95 ±0.2	11.09 ±0.2	10.95 ±0.2	10.62 ±0.2	11.27 ±0.1
All Groups	9.76 ±0.1	11.10 ±0.1	11.98 ±0.06	11.27 ±0.1	11.76 ±0.1	11.64 ±0.1	11.95 ±0.06	11.33 ±0.06	10.88 ±0.1	10.65 ±0.07	

Values are Mean ± S.E.

Significant difference within ages (<0.0001); Interaction between shed and treatments (0.0144), between shed and ages (<0.0001)

Table C16 Excreta moisture (%) of ISA brown following IB revaccination in different IB vaccination protocols in laying phase in birds that were not revaccinated after 14 weeks of age

Time of Revaccination	Week 14	Week 22		Week 30		Week 38		Week 46		Week 54	
Time of Excreta Collection	Week 16	Week 23	Week 24	Week 31	Week 32	Week 39	Week 40	Week 47	Week 48	Week 55	Week 56
Not revaccinated	68.85 ±1.1	73.28 ±0.6	73.69 ±0.9	77.31 ±0.6	78.12 ±1.0	71.76 ±0.6	70.74 ±0.5	70.34 ±1.0	69.24 ±0.8	69.94 ±1.0	72.19 ±0.8
Control	76.08 ±3.0	74.95 ±0.8	76.18 ±1.3	74.71 ±1.1	75.33 ±0.7	74.19 ±1.0	73.84 ±1.9	72.21 ±2.6	72.75 ±2.3	71.54 ±1.5	75.06 ±2.9
VicS, eyedrop at day-old	66.59 ±3.2	73.79 ±1.1	74.84 ±1.4	74.71 ±1.1	75.33 ±0.7	71.18 ±1.1	69.84 ±1.2	72.10 ±0.9	68.74 ±2.3	70.63 ±2.7	69.88 ±1.3
VicS, coarse spray at day-old	67.93 ±1.7	71.02 ±0.7	71.89 ±1.5	73.99 ±0.8	76.78 ±1.9	73.85 ±1.9	71.95 ±1.2	75.03 ±3.3	73.06 ±0.4	67.37 ±5.7	72.76 ±1.5
VicS, in water at day-old	71.84 ±1.7	73.48 ±0.5	74.71 ±1.06	73.99 ±0.9	74.92 ±1.3	69.23 ±1.2	70.27 ±1.2	68.51 ±1.2	68.28 ±1.6	70.09 ±2.5	71.97 ±1.9
A3, eyedrop at day-old	65.52 ±2.0	69.02 ±2.4	75.49 ±1.8	76.25 ±0.9	73.87 ±1.8	73.41 ±1.9	70.35 ±1.3	68.75 ±1.4	67.31 ±2.4	72.71 ±1.0	72.55 ±2.0
A3, coarse spray at day-old	63.02 ±1.9	73.60 ±0.9	67.28 ±5.6	73.74 ±1.1	74.80 ±0.4	70.09 ±0.7	70.94 ±0.5	69.89 ±1.9	68.68 ±1.4	70.20 ±1.4	72.97 ±1.3
A3, in water at day-old	70.93 ±3.1	77.07 ±2.0	75.73 ±1.6	70.94 ±0.7	77.69 ±1.1	70.39 ±1.3	68.00 ±0.8	65.88 ±4.2	65.87 ±2.1	67.05 ±1.6	70.12 ±2.8

Values are Mean ± S.E.

Table C16 (cont) Excreta moisture (%) of ISA brown following IB revaccination in different IB vaccination protocols in laying phase in birds that were revaccinated every 8 weeks during lay

Time of Revaccination	Week 14	Week 22		Week 30		Week 38		Week 46		Week 54	
Time of Excreta Collection	Week 16	Week 23	Week 24	Week 31	Week 32	Week 39	Week 40	Week 47	Week 48	Week 55	Week 56
Revaccinated	71.55 ±1.0	72.22 ±4.4	70.08 ±0.8	75.48 ±0.5	76.24 ±0.5	72.96 ±0.7	70.19 ±0.5	70.29 ±0.9	67.23 ±1.0	72.29 ±0.6	69.36 ±1.0
Control	73.39 ±3.9	69.50 ±1.0	69.79 ±2.3	71.44 ±1.4	74.28 ±1.9	69.73 ±1.7	67.77 ±0.8	71.68 ±0.9	62.88 ±1.9	69.93 ±1.1	63.26 ±3.0
VicS, eyedrop at day-old	68.21 ±3.2	73.85 ±1.6	68.08 ±3.9	75.67 ±0.9	74.70 ±2.3	73.18 ±2.5	70.53 ±0.7	72.40 ±2.9	67.03 ±2.6	69.83 ±2.4	70.87 ±2.2
VicS, coarse spray at day-old	68.51 ±0.5	69.22 ±2.1	68.61 ±2.0	74.64 ±0.6	76.60 ±0.3	71.71 ±1.5	68.04 ±1.6	66.97 ±1.4	62.46 ±2.9	71.35 ±0.7	68.79 ±2.1
VicS, in water at day-old	71.10 ±1.5	69.90 ±1.6	69.74 ±1.0	75.35 ±0.8	76.14 ±1.2	70.73 ±2.4	69.96 ±1.0	69.75 ±2.4	66.15 ±0.9	72.88 ±1.9	69.91 ±1.2
A3, eyedrop at day-old	75.74 ±2.8	76.55 ±2.1	71.32 ±1.6	77.01 ±0.9	76.81 ±1.5	75.90 ±1.5	72.65 ±2.0	69.85 ±4.7	69.96 ±2.5	74.62 ±1.4	71.63 ±4.7
A3, coarse spray at day-old	72.59 ±2.1	74.63 ±1.6	72.43 ±2.5	77.17 ±1.3	77.48 ±0.6	75.42 ±1.3	72.61 ±0.8	70.13 ±2.7	71.41 ±2.6	72.68 ±0.9	71.84 ±1.0
A3, in water at day-old	71.30 ±1.9	71.90 ±1.8	70.62 ±1.7	77.08 ±0.4	77.70 ±0.5	74.03 ±1.2	69.80 ±0.9	71.28 ±0.9	70.75 ±1.0	74.75 ±1.3	69.18 ±2.7

Values are Mean ± S.E.

Table C17 Haematocrit value (%) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

	Wk 16	Wk 27	Wk 35	Wk 43	Wk 49	Wk 58
Not revaccinated	29.83 ±1.0	30.23 ±0.3	31.63 ±0.4	30.36 ±0.5	32.07 ±0.3	29.00 ±0.2
Control	27.20 ±3.3	30.70 ±1.4	31.80 ±1.3	31.20 ±1.4	31.70 ±1.0	29.50 ±0.5
VicS, eyedrop at day-old	30.60 ±2.9	31.90 ±0.9	31.00 ±0.8	31.30 ±1.1	32.70 ±1.7	29.80 ±0.2
VicS, coarse spray at day-old	24.40 ±1.2	29.20 ±1.0	31.40 ±0.7	29.40 ±1.1	31.20 ±0.7	29.20 ±0.6
VicS, in water at day-old	33.10 ±1.5	29.50 ±1.0	31.60 ±1.0	30.80 ±1.6	32.20 ±0.9	28.20 ±1.0
A3, eyedrop at day-old	31.70 ±0.7	30.10 ±0.8	31.40 ±0.7	28.50 ±2.7	32.30 ±0.8	28.90 ±0.4
A3, coarse spray at day-old	35.00 ±3.5	30.20 ±0.2	30.40 ±0.7	30.80 ±1.1	32.60 ±0.5	28.50 ±0.8
A3, in water at day-old	26.80 ±2.1	30.00 ±0.9	33.80 ±1.6	30.50 ±0.6	31.80 ±1.2	28.90 ±0.7
Revaccinated	28.47 ±1.2	30.00 ±0.4	31.40 ±0.3	30.80 ±0.5	31.70 ±0.3	29.16 ±0.4
Control	24.20 ±2.5	31.80 ±1.5	32.20 ±0.6	33.30 ±1.2	32.20 ±0.7	29.60 ±0.9
VicS, eyedrop at day-old	27.10 ±2.1	29.80 ±0.5	31.00 ±0.5	31.30 ±0.9	31.60 ±0.8	29.40 ±1.3
VicS, coarse spray at day-old	22.80 ±2.4	31.30 ±0.7	31.30 ±0.7	29.00 ±0.9	32.20 ±0.6	31.00 ±1.5
VicS, in water at day-old	35.00 ±5.6	28.40 ±1.0	29.40 ±0.7	27.80 ±1.9	31.20 ±0.8	27.10 ±0.9
A3, eyedrop at day-old	29.90 ±1.2	30.00 ±1.3	32.60 ±0.9	29.80 ±0.5	30.90 ±0.6	29.40 ±0.8
A3, coarse spray at day-old	31.90 ±1.1	30.20 ±1.7	31.40 ±0.9	32.50 ±0.5	31.50 ±0.6	29.40 ±0.9
A3, in water at day-old	28.40 ±1.3	28.90 ±0.7	31.90 ±1.3	31.90 ±1.3	32.30 ±1.2	28.20 ±0.6

Values are Mean ± S.E.
Significant difference among ages (<0.0001);
Interaction between ages and initial treatments (<0.0001)

Table C18 Plasma sodium Na^+ (mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

	Wk 16	Wk 27	Wk 35	Wk 43	Wk 49	Wk 58
Not revaccinated	146.37 ±0.5	147.10 ±0.4	147.10 ±0.4	148.89 ±0.5	152.74 ±0.4	136.25 ±0.8
Control	146.68 ±1.5	145.40 ±1.0	151.44 ±1.8	150.08 ±2.1	152.74 ±0.6	152.74 ±0.5
VicS, eyedrop at day-old	145.42 ±0.73	146.82 ±0.8	153.16 ±1.0	149.36 ±1.7	151.66 ±1.2	135.32 ±3.7
VicS, coarse spray at day-old	147.96 ±1.3	147.18 ±1.7	152.10 ±0.9	150.10 ±1.3	153.78 ±1.2	133.92 ±2.3
VicS, in water at day-old	143.84 ±2.4	147.86 ±0.4	152.48 ±0.8	148.04 ±0.6	151.04 ±1.4	135.94 ±2.1
A3, eyedrop at day-old	146.70 ±0.4	147.38 ±1.0	152.02 ±1.3	149.36 ±1.8	154.94 ±1.5	139.14 ±1.6
A3, coarse spray at day-old	146.14 ±1.1	147.62 ±0.7	151.94 ±0.3	148.48 ±1.1	152.70 ±0.4	135.86 ±1.5
A3, in water at day-old	147.88 ±1.3	147.42 ±1.0	151.62 ±1.5	146.80 ±1.3	152.82 ±0.6	136.88 ±0.8
Revaccinated	146.81 ±0.4	147.87 ±0.4	152.19 ±0.4	149.93 ±0.5	152.57 ±0.5	134.79 ±0.8
Control	146.46 ±1.7	145.68 ±0.7	150.58 ±0.9	148.76 ±1.6	152.24 ±1.1	134.02 ±2.4
VicS, eyedrop at day-old	145.68 ±1.2	147.10 ±1.6	152.58 ±1.0	148.36 ±0.7	152.14 ±1.0	133.44 ±2.3
VicS, coarse spray at day-old	147.60 ±1.1	148.68 ±0.9	150.74 ±1.1	151.40 ±1.9	153.00 ±0.8	133.74 ±1.3
VicS, in water at day-old	146.88 ±0.7	148.18 ±1.0	152.04 ±0.7	149.74 ±1.9	152.76 ±2.1	133.00 ±2.8
A3, eyedrop at day-old	146.56 ±1.0	149.02 ±0.5	151.20 ±1.1	150.08 ±1.0	152.26 ±2.3	133.36 ±1.8
A3, coarse spray at day-old	146.58 ±0.9	147.10 ±1.2	154.30 ±1.4	149.52 ±1.0	154.88 ±1.2	139.18 ±0.7
A3, in water at day-old	147.92 ±1.2	149.12 ±0.5	153.90 ±0.4	151.66 ±1.9	152.18 ±1.2	136.80 ±0.8

Values are Mean ± S.E.
Significant difference within ages (<0.0001)

Table C19 Plasma potassium K^+(mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

	Wk 16	Wk 27	Wk 35	Wk 43	Wk 49	Wk 58
Not revaccinated	5.75 ±0.1	4.93 ±0.1	5.36 ±0.1	5.29 ±0.1	5.06 ±0.1	5.35 ±0.1
Control	5.45 ±0.1	4.64 ±0.2	5.14 ±0.2	5.00 ±0.2	5.29 ±0.2	5.67 ±0.1
VicS, eyedrop at day-old	5.62 ±0.1	4.75 ±0.1	5.13 ±0.1	5.12 ±0.2	5.09 ±0.2	5.46 ±0.3
VicS, coarse spray at day-old	6.06 ±0.2	4.91 ±0.3	4.85 ±0.1	4.94 ±0.03	5.08 ±0.2	5.25 ±0.1
VicS, in water at day-old	5.81 ±0.1	5.03 ±0.3	5.53 ±0.3	5.52 ±0.3	5.21 ±0.2	5.36 ±0.2
A3, eyedrop at day-old	5.62 ±0.1	5.04 ±0.2	5.53 ±0.3	5.63 ±0.2	5.22 ±0.1	5.09 ±0.2
A3, coarse spray at day-old	5.62 ±0.1	5.04 ±0.2	5.53 ±0.3	5.63 ±0.2	5.22 ±0.2	5.09 ±0.2
A3, in water at day-old	5.83 ±0.2	4.93 ±0.2	5.55 ±0.03	5.47 ±0.2	9.95 ±0.2	5.20 ±0.1
Revaccinated	5.92 ±0.1	5.07 ±0.1	5.40 ±0.1	5.33 ±0.1	4.96 ±0.1	5.43 ±0.1
Control	5.61 ±0.2	4.64 ±0.1	5.06 ±0.2	5.24 ±0.3	4.84 ±0.1	5.53 ±0.1
VicS, eyedrop at day-old	5.66 ±0.1	5.12 ±0.3	5.06 ±0.2	4.95 ±0.1	4.90 ±0.2	5.22 ±0.05
VicS, coarse spray at day-old	6.01 ±0.4	5.02 ±0.1	4.68 ±0.1	5.13 ±0.1	5.16 ±0.2	5.46 ±0.2
VicS, in water at day-old	6.06 ±0.1	5.04 ±0.2	5.03 ±0.1	5.14 ±0.1	4.84 ±0.2	5.42 ±0.3
A3, eyedrop at day-old	5.90 ±0.2	5.15 ±0.1	5.90 ±0.1	5.60 ±0.3	4.95 ±0.2	5.38 ±0.3
A3, coarse spray at day-old	6.17 ±0.1	5.27 ±0.1	6.03 ±0.2	5.81 ±0.2	4.95 ±0.1	5.45 ±0.1
A3, in water at day-old	603 ±0.2	5.27 ±0.1	6.01 ±0.1	5.47 ±0.3	5.11 ±0.1	5.53 ±0.1

Values are Mean ± S.E.
Significant difference among initial treatments (.0029), and among ages (<0.0001); Interaction between ages and initial treatments (<0.0001)

Table C20 Plasma Ca^{++} (mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

	Wk 16	Wk 27	Wk 35	Wk 43	Wk 49	Wk 58
Not revaccinated	1.48 ±0.03	1.57 ±0.02	1.73 ±0.02	1.62 ±0.02	1.72 ±0.02	1.25 ±0.02
Control	1.48 ±0.05	1.55 ±0.08	1.73 ±0.05	1.69 ±0.05	1.74 ±0.03	1.18 ±0.02
VicS, eyedrop at day-old	1.38 ±0.02	1.54 ±0.06	1.73 ±0.04	1.58 ±0.04	1.61 ±0.04	1.20 ±0.13
VicS, coarse spray at day-old	1.63 ±0.11	1.49 ±0.05	1.69 ±0.03	1.66 ±0.02	1.76 ±0.05	1.25 ±0.04
VicS, in water at day-old	1.36 ±0.04	1.63 ±0.08	1.77 ±0.04	1.63 ±0.05	1.76 ±0.06	1.30 ±0.03
A3, eyedrop at day-old	1.51 ±0.07	1.56 ±0.04	1.69 ±0.05	1.63 ±0.07	1.73 ±0.03	1.30 ±0.02
A3, coarse spray at day-old	1.50 ±0.10	1.65 ±0.05	1.77 ±0.06	1.61 ±0.05	1.78 ±0.05	1.29 ±0.02
A3, in water at day-old	1.50 ±0.03	1.55 ±0.04	1.75 ±0.01	1.57 ±0.05	1.65 ±0.07	1.27 ±0.04
Revaccinated	1.49 ±0.02	1.57 ±0.02	1.73 ±0.02	1.63 ±0.02	1.74 ±0.02	1.26 ±0.02
Control	1.50 ±0.08	1.57 ±0.07	1.70 ±0.06	1.61 ±0.09	1.71 ±0.07	1.26 ±0.02
VicS, eyedrop at day-old	1.41 ±0.03	1.51 ±0.03	1.69 ±0.05	1.62 ±0.04	1.79 ±0.04	1.25 ±0.01
VicS, coarse spray at day-old	1.62 ±0.07	1.49 ±0.02	1.60 ±0.07	1.58 ±0.05	1.75 ±0.06	1.19 ±0.07
VicS, in water at day-old	1.50 ±0.09	1.59 ±0.04	1.81 ±0.04	1.61 ±0.07	1.75 ±0.07	1.26 ±0.03
A3, eyedrop at day-old	1.43 ±0.03	1.59 ±0.02	1.73 ±0.06	1.63 ±0.06	1.76 ±0.06	1.25 ±0.04
A3, coarse spray at day-old	1.46 ±0.04	1.64 ±0.05	1.79 ±0.05	1.67 ±0.02	1.72 ±0.06	1.32 ±0.06
A3, in water at day-old	1.53 ±0.06	1.60 ±0.04	1.78 ±0.02	1.70 ±0.05	1.71 ±0.03	1.29 ±0.04

Values are Mean ± S.E.
Significant difference among ages ($P<0.0001$)

Table C21 IBV antibody titres of ISA brown hens exposed to the following different IB vaccination protocols in laying phase

	Wk 16	Wk 27	Wk 35	Wk 43	Wk 49	Wk 58
Not revaccinated	1599.74 ±297.8	680.66 ±183.2	621.09 ±160.7	559.11 ±169.1	767.97 ±141.2	423.66 ±107.8
Control	2937.20 ±1130.8	1346.20 ±748.8	1358.20 ±727.4	1243.80 ±623.6	973.60 ±424.9	962.20 ±484.0
VicS, eyedrop at day-old	859.60 ±580.4	161.20 ±85.8	544.20 ±242.9	275.20 ±71.0	791.80 ±321.7	103.20 ±44.4
VicS, coarse spray at day-old	1842.20 ±945.7	1114.20 ±772.0	943.40 ±682.7	1170.00 ±957.2	1052.40 ±731.4	648.40 ±493.2
VicS, in water at day-old	1065.80 ±856.0	370.00 ±218.8	256.80 ±137.3	162.00 ±99.9	269.20 ±132.4	124.60 ±76.7
A3, eyedrop at day-old	2351.00 ±712.5	1262.80 ±570.8	864.60 ±383.6	678.00 ±174.5	1032.00 ±364.1	470.80 ±121.6
A3, coarse spray at day-old	814.40 ±237.0	370.20 ±160.2	216.40 ±103.6	227.40 ±87.5	543.80 ±158.2	449.40 ±208.6
A3, in water at day-old	1328.00 ±696.8	140.00 ±56.0	164.00 ±27.8	157.40 ±36.9	713.00 ±261.7	207.00 ±47.1
Revaccinated	1314.61 ±261.1	403.61 ±79.6	864.12 ±397.7	464.82 ±85.7	493.12 ±78.6	303.58 ±58.0
Control	1495.40 ±414.3	333.80 ±122.6	334.60 ±114.7	410.00 ±99.9	617.80 ±284.9	306.40 ±109.6
VicS, eyedrop at day-old	595.40 ±306.9	226.80 ±96.4	305.20 ±142.8	335.00 ±173.0	403.20 ±180.4	86.00 ±30.1
VicS, coarse spray at day-old	1442.20 ±594.3	535.00 ±301.9	603.20 ±293.4	664.40 ±322.6	562.80 ±223.6	673.60 ±253.1
VicS, in water at day-old	624.40 ±287.8	87.60 ±25.6	2729.20 ±2602.4	153.20 ±80	320.80 ±140.6	123.80 ±61.4
A3, eyedrop at day-old	873.50 ±284.0	513.75 ±126.1	350.75 ±19.0	400.50 ±75.5	418.50 ±161.0	301.00 ±63.3
A3, coarse spray at day-old	3178.00 ±1252.1	804.00 ±347.2	1172.60 ±537.9	624.60 ±348.1	640.00 ±266.1	410.40 ±180.2
A3, in water at day-old	802.75 ±317.6	332.00 ±102.0	347.25 ±87.4	700.25 ±303.7	469.00 ±242.5	203.25 ±73.4

Values are Mean ± S.E.
Significant difference within ages (<0.0001)

Appendix D: Trial 1 – Moulting and Challenge Phase

Table D1 Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase (**moulting before vaccination**)

Week	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73
Not revaccinated																	
Control	63.7 ±2.1	12.1 ±3.5	21.0 ±3.9	40.8 ±1.9	19.4 ±5.2	28.1 ±5.2	55.7 ±4.8	75.1 ±6.1	77.1 ±6.7	74.1 ±5.8	75.2 ±8.3	46.2 ±14.4	51.6 ±11.6	69.6 ±2.6	82.9 ±2.0	78.8 ±3.4	68.3 ±2.0
VicS, eyedrop at day-old	57.9 ±3.7	11.4 ±3.9	11.6 ±2.3	40.3 ±2.7	11.2 ±1.7	19.4 ±4.5	54.2 ±6.5	71.2 ±7.5	70.8 ±4.5	69.6 ±5.2	82.7 ±6.9	75.6 ±3.4	66.4 ±3.9	58.6 ±4.7	62.5 ±10.3	75.6 ±5.4	68.8 ±4.0
VicS, coarse spray at day-old	59.7 ±5.8	21.0 ±5.7	20.1 ±3.1	46.4 ±12	25.7 ±8.4	24.2 ±2.8	67.0 ±5.9	88.3 ±7.2	98.0 ±3.9	76.7 ±2.5	90.7 ±6.0	86.7 ±4.3	81.7 ±3.1	74.8 ±6.8	88.1 ±4.2	91.2 ±4.9	80.8 ±4.7
VicS, in water at day-old	63.8 ±7.5	7.5 ±2.9	5.4 ±1.9	45.0 ±9.2	22.5 ±3.9	23.6 ±3.6	56.2 ±5.6	77.9 ±2.3	74.4 ±6.1	67.4 ±4.4	82.5 ±4.6	71.8 ±7.5	75.00 ±4.7	73.6 ±3.4	72.1 ±5.4	76.4 ±7.6	82.2 ±7.6
A3, eyedrop at day-old	61.5 ±6.0	5.0 ±4.0	9.1 ±2.0	17.2 ±2.3	20.4 ±2.2	30.1 ±4.4	70.5 ±4.1	88.8 ±1.3	85.2 ±3.6	84.6 ±3.8	93.0 ±5.3	91.1 ±3.6	95.12 ±0.8	85.2 ±3.9	89.9 ±3.4	91.1 ±3.6	87.5 ±8.3
A3, coarse spray at day-old	62.5 ±3.0	5.6 ±1.7	2.6 ±1.5	25.0 5.5	22.6 ±6.0	23.0 ±2.2	65.5 ±5.1	81.7 ±9.7	86.0 ±3.3	76.8 ±5.8	82.0 ±8.4	80.6 ±4.5	88.21 ±4.8	68.6 ±5.3	81.0 ±6.7	76.1 ±8.3	72.9 ±12
A3, in water at day-old	69.3 ±1.9	11.7 ±4.2	18.8 ±2.0	24.0 ±5.4	13.0 ±5.5	30.1 ±3.2	69.0 ±7.3	95.8 ±3.0	83.6 ±3.1	82. ±3.3	93.7 ±1.3	91.0 ±3.5	83.57 ±4.3	81.9 ±1.5	90.5 ±1.9	90.2 ±2.0	86.9 ±7.9

Table D1 (Cont) Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase (**moulting before vaccination**)

Week	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73
Revaccinated																	
Control	47.5 ±4.8	11.9 ±5.9	15.2 ±4.5	28.89 ±8.2	10.0 ±1.6	25.6 ±6.5	58.0 ±5.5	82.6 ±5.6	77.9 ±3.1	75.5 ±5.5	84.4 ±4.3	70.6 ±5.2	74.3 ±3.0	73.6 ±4.7	80.0 ±7.7	80.0 ±7.7	54.0 ±4.1
VicS, eyedrop at day-old	50.5 ±2.4	14.4 ±5.7	21.2 ±4.1	35.56 ±8.6	15.0 ±5.2	29.5 ±3.2	70.0 ±3.7	84.3 ±3.0	84.3 ±3.8	78.1 ±1.4	93.0 ±2.1	87.1 ±1.7	80.2 ±4.9	76.0 ±4.3	86.7 ±2.8	86.7 ±2.8	59.0 ±5.8
VicS, coarse spray at day-old	54.8 ±4.4	20.4 ±6.9	20.0 ±1.9	50.00 ±9.6	24.2 ±6.4	26.4 ±9.0	73.0 ±4.7	83.3 ±9.6	84.9 ±4.6	71.7 ±3.2	88.6 ±8.3	79.5 ±3.0	70.7 ±5.1	62.6 ±4.0	74.9 ±3.9	74.9 ±3.9	45.0 ±3.7
VicS, in water at day-old	46.5 ±7.0	14.4 ±6.5	12.6 ±4.9	43.06 ±3.4	19.3 ±4.2	22.9 ±2.6	59.8 ±4.8	73.8 ±7.2	74.6 ±7.8	61.8 ±6.4	84.5 ±5.9	71.0 ±7.2	72.9 ±6.2	69.2 ±3.7	68.8 ±8.2	68.8 ±8.2	54.4 ±3.0
A3, eyedrop at day-old	50.7 ±4.3	12.5 ±4.3	22.3 ±4.9	53.33 ±3.2	16.9 ±3.6	23.5 ±1.9	70.1 ±1.6	81.1 ±6.7	82.6 ±0.6	73.0 ±5.2	78.8 ±4.0	79.5 ±2.0	82.6 ±5.4	64.5 ±4.8	64.1 ±4.3	64.1 ±4.3	44.2 ±7.1
A3, coarse spray at day-old	45.8 ±4.2	24.6 10.9	17.7 ±8.9	25.83 ±6.4	19.6 ±7.0	29.4 ±6.9	62.4 ±2.3	88.9 ±4.4	82.4 ±1.3	76.3 ±5.3	87.5 ±2.7	84.3 ±0.8	83.7 ±5.4	76.2 ±0.7	78.7 ±1.9	78.7 ±1.9	55.4 ±7.6
A3, in water at day-old	53.9 ±7.3	12.9 ±2.2	10.4 ±2.0	43.47 ±16.5	17.1 ±3.8	19.7 ±2.5	65.6 ±4.3	87.8 ±3.4	82.9 ±7.1	66.6 ±5.6	87.4 ±10.9	80.2 ±9.4	78.7 ±8.0	73.8 ±8.8	76.5 ±7.9	76.5 ±7.9	47.8 ±7.2

Values are Mean ± S.E.

Significant difference within sheds (0.0010), ages (<0.0001), initial treatments (<0.0001); Interaction between shed and ages (<0.0001), between shed and treatment (<0.0001), treatment and ages (0.0410), between shed, ages and treatment (0.0229)

Table D2 Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase (**moulting after vaccination**)

Week	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73
Not revaccinated																	
Control	81.5 ±13.5	75.1 ±10.3	86.0 ±8.8	114 ±1.1	89.4 ±2.7	60.1 ±5.9	8.7 ±3.9	11.4 ±4.6	11.4 ±2.7	6.4 ±1.6	14.1 ±5.0	62.9 ±10.3	67.7 ±7.2	72.3 ±4.8	73.9 ±3.3	7879 ±8.3	74.2 ±5.5
VicS, eyedrop at day-old	85.3 ±9.7	70.0 ±4.0	82.9 ±7.9	86.9 ±7.3	76.7 5.1	52.5 ±5.6	6.0 ±2.1	6.6 ±4.9	10.9 ±3.2	0.9 ±0.9	18.1 ±8.3	52.3 ±7.6	60.6 ±7.8	67.9 ±2.1	78.5 ±6.7	75.7 ±5.4	74.0 ±2.6
VicS, coarse spray at day-old	86.6 ±11.7	78.7 ±6.6	78.9 ±4.6	109 ±12.9	83.0 ±8.7	61.6 ±6.4	17.9 ±4.4	20.14 ±4.9	12.5 ±4.4	5.1 ±2.9	21.4 ±5.1	67.6 ±10.2	72.9 ±3.0	77.4 ±6.4	91.7 ±4.4	91.1 ±2.3	89.1 ±1.6
VicS, in water at day-old	97.9 ±7.2	81.7 ±7.8	90.7 ±7.9	103 ±5.8	85.7 7.9	57.0 ±4.8	16.6 ±4.9	23. ±4.7	13.0 ±4.4	2.9 ±0.3	29.6 ±4.5	61.7 ±6.5	71.6 ±6.7	72.9 ±3.6	76.5 ±4.1	74.6 ±9.8	77.4 ±12.2
A3, eyedrop at day-old	100.7 ±2.1	80.8 ±8.5	89.7 ±7.5	105 ±15.0	89.4 ±5.7	67.3 ±3.0	25.6 ±4.8	18.3 ±10.8	8.9 ±3.6	3.0 ±2.0	24.8 ±1.8	74.2 ±4.8	73.0 ±7.4	60.1 ±8.5	73.3 ±9.0	78.5 ±6.5	77.1 ±8.1
A3, coarse spray at day-old	97.3 ±6.5	82.9 ±3.4	88.6 ±6.2	97.8 ±9.3	80.4 ±2.0	60.5 ±2.1	22.4 ±4.2	20.1 ±2.5	8.5 ±1.9	3.3 ±1.3	14.2 ±3.0	68.9 ±2.4	74.9 ±5.0	78.9 ±2.7	85.5 ±2.9	84.7 ±4.1	82.3 ±5.0
A3, in water at day-old	91.0 ±4.8	75.4 ±5.6	86.1 ±7.7	102 ±11.4	84.9 ±7.7	67.4 ±4.0	33.4 ±4.1	24.7 ±5.0	6.6 ±3.1	10.1 ±2.5	15.1 ±3.8	80.1 ±5.2	83.6 ±6.4	69.6 ±4.5	81.6 ±3.4	85.3 ±1.8	75.0 ±6.7

Table D2(Cont) Hen-day egg production (%) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase (**moulting after vaccination**)

Week	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73
Revaccinated																	
Control	85.8 ±8.4	87.4 ±9.8	85.5 ±13.6	96.7 ±8.1	79.0 ±7.1	55.0 ±2.1	9.1 ±2.3	8.7 ±5.2	6.0 ±2.4	5.8 ±2.0	19.3 ±9.5	60.1 ±12.0	78.5 ±5.5	78.4 ±7.5	85.3 ±9.0	85.3 ±9.0	63.1 ±5.1
VicS, eyedrop at day-old	79.3 ±4.4	80.7 ±8.4	96.7 ±7.0	103.6 ±6.5	77.6 ±4.7	69.9 ±4.6	17.9 ±3.6	15.0 ±4.3	10.0 ±3.5	4.3 ±1.8	26.2 ±10.6	58.8 ±4.0	72.0 ±6.5	73.3 ±3.0	76.2 ±5.6	76.2 ±5.6	57.9 ±5.7
VicS, coarse spray at day-old	83.8 ±6.0	80.2 ±3.4	92.3 ±2.0	108.3 ±3.2	81.7 ±2.9	58.2 ±3.3	10.0 ±1.8	14.6 ±2.5	7.9 ±3.6	5.0 ±1.8	18.8 ±4.1	63.6 ±12.9	80.5 ±9.2	75.2 ±5.0	78.8 ±11.0	78.8 ±11.0	63.4 ±6.9
VicS, in water at day-old	88.8 ±4.3	83.4 ±2.9	89.3 ±2.4	121.5 ±7.0	83.4 ±3.5	67.1 ±2.7	22.9 ±5.2	25.0 ±6.8	9.3 ±2.7	8.6 ±2.3	30.0 ±8.1	82.9 ±3.5	82.1 ±3.2	81.4 ±1.8	85.0 ±2.9	85.0 ±2.9	61.3 ±4.3
A3, eyedrop at day-old	88.3 ±4.8	88.9 ±4.4	96.4 ±2.1	107.8 ±8.5	84.4 ±3.1	66.9 ±4.2	22.5 ±4.4	14.7 ±6.1	3.6 ±1.6	7.1 ±0.6	26.4 ±1.4	66.1 ±7.4	80.8 ±2.1	75.0 ±2.1	86.8 ±3.0	86.8 ±3.0	60.4 ±3.7
A3, coarse spray at day-old	76.1 ±9.8	87.0 ±10.1	93.6 ±8.4	108.7 ±12.3	86.0 ±10.6	69.8 ±9.5	19.5 ±8.0	11.6 ±4.7	4.3 ±1.1	8.7 ±2.5	22.5 ±7.1	66.3 ±7.7	73.3 ±7.7	67.1 ±6.9	71.6 ±15.0	71.6 ±15.0	42.7 ±6.5
A3, in water at day-old	85.8 ±5.2	77.5 ±3.9	91.4 ±4.4	105.6 ±3.2	88.5 ±2.7	59.8 ±4.1	8.5 ±4.3	5.7 ±4.8	12.7 ±4.8	4.9 ±1.3	22.1 ±5.4	70.1 ±5.3	81.5 ±1.8	80.3 ±4.9	81.4 ±3.6	81.4 ±3.6	61.0 ±3.8

Values are Mean ± S.E.

Significant difference within ages (<0.0001), initial treatments (<0.0001); Interaction between shed and ages (<0.0001), between shed and treatment (0.0038)

Table D3 Egg quality egg weight (g) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		Moulting after revaccination	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	62.31 ±1.2	61.01 ±3.5	63.68 ±1.4	62.57 ±1.2	65.79 ±1.3	63.19 ±1.1	64.54 ±0.9
VicS, eyedrop at day-old	64.62 ±1.5	63.17 ±0.9	63.21 ±1.2	62.45 ±0.6	65.57 ±1.7	64.41 ±1.0	66.21 ±1.9
VicS, coarse spray at day-old	63.07 ±1.4	65.07 ±1.2	63.81 ±1.2	66.88 ±1.0	65.78 ±1.2	63.30 ±1.3	62.23 ±1.3
VicS, in water at day-old	62.40 ±0.8	62.42 ±0.8	63.21 ±0.9	63.74 ±0.8	65.50 ±1.5	65.54 ±1.1	63.78 ±1.8
A3, eyedrop at day-old	62.99 ±1.1	62.38 ±1.0	63.92 ±0.9	65.86 ±1.1	61.85 ±1.1	65.07 ±1.2	64.59 ±2.4
A3, coarse spray at day-old	65.04 ±1.0	65.28 ±1.2	65.28 ±1.0	66.55 ±1.0	67.94 ±1.5	66.29 ±0.9	64.05 ±1.6
A3, in water at day-old	62.79 ±0.9	63.32 ±0.7	61.61 ±0.6	64.26 ±0.8	64.26 ±1.3	65.49 ±0.9	65.77 ±1.0
Revaccinated							
Control	63.65 ±1.0	65.76 ±1.2	65.89 ±1.0	65.09 ±0.8	64.37 ±1.5	63.59 ±0.8	64.35 ±1.6
VicS, eyedrop at day-old	65.08 ±1.3	67.29 ±1.4	65.79 ±1.0	64.88 ±0.8	67.15 ±1.4	66.93 ±1.5	65.14 ±2.2
VicS, coarse spray at day-old	60.21 ±4.2	65.41 ±1.3	63.89 ±1.5	65.27 ±1.5	64.79 ±1.9	64.87 ±0.9	66.90 ±1.5
VicS, in water at day-old	63.60 ±1.2	64.54 ±1.1	65.32 ±0.9	64.81 ±0.8	68.95 ±1.3	64.24 ±1.2	64.77 ±1.7
A3, eyedrop at day-old	63.85 ±1.2	65.48 ±1.2	64.14 ±1.2	64.47 ±0.8	65.59 ±1.6	64.53 ±1.2	62.18 ±1.2
A3, coarse spray at day-old	60.11 ±1.0	65.17 ±1.3	62.47 ±3.4	64.90 ±0.8	64.42 ±1.6	62.87 ±0.8	63.78 ±1.3
A3, in water at day-old	63.58 ±0.8	64.36 ±1.2	64.81 ±1.5	66.16 ±1.7	65.72 ±1.5	64.39 ±1.0	65.22 ±1.1

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, significant difference within shed (0.0044); 68 wks, no significant difference; 72 wks, interaction between shed and initial treatments (0.0264); 78 wks, no significant difference

Table D4 Egg quality shell reflectivity (%) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		**Moulting after revaccination**	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	35.76 ±1.3	32.11 ±1.0	35.84 ±1.9	33.00 ±0.9	36.40 ±2.0	31.50 ±0.9	33.50 ±1.6
VicS, eyedrop at day-old	34.00 ±1.0	30.83 ±0.9	30.60 ±1.1	30.95 ±0.9	35.40 ±3.0	30.10 ±1.0	29.50 ±0.5
VicS, coarse spray at day-old	35.75 ±1.3	32.71 ±1.0	32.67 ±1.0	31.50 ±1.0	35.50 ±2.2	34.00 ±1.2	32.70 ±1.5
VicS, in water at day-old	33.29 ±0.7	32.42 ±0.8	30.28 ±0.8	32.10 ±0.7	36.20 ±2.9	30.70 ±0.8	31.80 ±1.6
A3, eyedrop at day-old	34.48 ±1.2	32.86 ±1.2	29.43 ±0.8	31.05 ±0.9	34.00 ±1.4	32.45 ±1.2	30.90 ±1.6
A3, coarse spray at day-old	36.91 ±1.2	32.37 ±1.1	28.79 ±1.0	31.10 ±0.6	38.50 ±2.9	32.60 ±1.1	31.40 ±1.7
A3, in water at day-old	37.19 ±1.1	31.10 ±0.7	31.19 ±0.9	31.90 ±0.8	34.80 ±1.7	33.05 ±1.1	32.89 ±1.4
Revaccinated							
Control	35.33 ±1.4	32.95 ±1.2	33.80 ±1.0	33.25 ±1.0	36.70 ±0.9	31.90 ±1.0	31.70 ±1.2
VicS, eyedrop at day-old	35.79 ±1.1	31.79 ±0.8	32.10 ±0.9	32.55 ±0.9	33.30 ±1.6	33.00 ±1.3	29.40 ±1.0
VicS, coarse spray at day-old	36.00 ±1.4	30.69 ±1.2	31.05 ±1.0	31.35 ±1.1	36.00 ±3.8	33.20 ±1.1	31.60 ±1.5
VicS, in water at day-old	36.81 ±1.0	33.42 ±1.1	30.52 ±0.8	32.00 ±1.1	38.40 ±3.2	32.40 ±0.8	34.30 ±1.0
A3, eyedrop at day-old	33.62 ±1.1	32.42 ±1.3	31.30 ±0.9	32.80 ±0.9	36.00 ±2.9	31.80 ±0.8	32.40 ±1.8
A3, coarse spray at day-old	36.81 ±0.9	32.65 ±0.7	30.32 ±0.7	32.90 ±0.9	34.10 ±2.3	31.70 ±0.8	31.90 ±1.2
A3, in water at day-old	35.30 ±1.2	33.00 ±0.8	32.76 ±0.7	33.75 ±0.8	33.90 ±1.7	30.70 ±0.9	32.30 ±2.1

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, no significant difference; 68 wks, significant difference within initial treatment (<0.0001); 72 wks, significant difference within initial treatment (0.0271), interaction of shed, initial treatment and moulting (0.0185); 78 wks, significant difference within moulting (0.0007), interaction between initial treatment and moulting (0.0435)

Table D5 Egg quality shell breaking strength (Newton) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		Moulting after revaccination	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	31.57 ±2.3	37.09 ±2.4	32.58 ±3.4	34.65 ±2.1	35.25 ±2.3	40.04 ±1.4	48.26 ±1.4
VicS, eyedrop at day-old	28.95 ±2.0	37.38 ±1.9	39.18 ±1.8	28.66 ±2.3	37.46 ±3.3	35.83 ±1.8	40.40 ±4.1
VicS, coarse spray at day-old	27.72 ±2.2	33.91 ±2.3	34.85 ±2.4	31.80 ±1.9	35.87 ±3.5	32.85 ±2.8	36.16 ±1.8
VicS, in water at day-old	30.71 ±1.7	39.20 ±2.3	35.01 ±2.7	36.28 ±2.4	37.08 ±2.6	36.84 ±2.2	40.06 ±2.4
A3, eyedrop at day-old	32.16 ±2.4	34.14 ±2.4	37.79 ±2.2	33.49 ±2.2	39.35 ±1.4	36.94 ±2.9	38.34 ±3.6
A3, coarse spray at day-old	31.01 ±2.7	34.44 ±2.4	39.58 ±2.5	35.45 ±1.9	34.91 ±2.9	37.17 ±2.2	40.95 ±3.6
A3, in water at day-old	34.81 ±2.8	32.05 ±2.2	35.65 ±2.6	36.91 ±1.5	39.71 ±2.8	36.61 ±2.0	42.66 ±4.0
Revaccinated							
Control	29.71 ±2.8	33.64 ±3.0	35.28 ±2.9	39.45 ±1.5	35.63 ±4.1	36.90 ±1.6	50.38 ±3.4
VicS, eyedrop at day-old	28.15 ±2.3	36.56 ±1.6	36.19 ±1.8	33.80 ±1.8	38.45 ±2.5	34.92 ±2.8	35.41 ±2.0
VicS, coarse spray at day-old	31.41 ±1.9	30.94 ±3.2	32.62 ±2.4	28.39 ±2.2	32.13 ±3.6	36.93 ±2.5	36.63 ±2.2
VicS, in water at day-old	32.96 ±1.9	38.43 ±2.3	33.35 ±2.5	34.55 ±2.0	38.87 ±3.1	34.69 ±2.3	32.75 ±4.3
A3, eyedrop at day-old	35.12 ±1.7	37.87 ±2.1	34.65 ±2.4	31.81 ±2.4	38.87 ±2.4	36.64 ±2.6	35.87 ±3.3
A3, coarse spray at day-old	35.84 ±2.4	38.43 ±1.8	33.29 ±2.2	35.58 ±1.8	32.83 ±3.5	39.51 ±1.9	34.04 ±4.6
A3, in water at day-old	29.92 ±2.6	36.83 ±2.7	36.96 ±2.7	39.80 ±2.4	41.93 ±2.4	41.70 ±1.2	36.43 ±3.9

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, no significant difference; 68 wks, no significant difference; 72 wks, significant difference within initial treatment (0.0001), moulting (0.0011); 78 wks, significant difference within initial treatment (0.0202), interaction between initial treatment and moulting (0.0051)

Table D6 Egg quality shell deformation (mm) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		**Moulting after revaccination**	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	261.43 ±29.1	253.33 ±17.6	302.94 ±35.2	282.50 ±36.9	277.00 ±33.6	262.50 ±23.7	295.00 ±10.6
VicS, eyedrop at day-old	237.50 ±19.8	251.67 ±21.8	263.50 ±19.95	315.50 ±42.5	249.00 ±31.1	249.00 ±11.9	273.00 ±26.1
VicS, coarse spray at day-old	281.50 ±29.9	311.43 ±44.0	257.14 ±24.6	244.50 ±28.8	213.00 ±14.1	226.50 ±9.6	227.00 ±8.2
VicS, in water at day-old	281.91 ±31.7	258.42 ±15.1	258.89 ±42.4	264.00 ±21.7	244.00 ±16.0	268.50 ±20.9	278.00 ±21.3
A3, eyedrop at day-old	267.14 ±18.7	251.50 ±18.8	274.76 ±35.4	312.50 ±33.6	235.00 ±15.3	314.00 ±29.1	390.00 ±72.2
A3, coarse spray at day-old	256.67 ±19.9	260.53 ±23.0	261.05 ±17.3	264.50 ±36.0	312.00 ±58.7	295.50 ±29.5	271.00 ±15.5
A3, in water at day-old	286.67 ±29.3	281.91 ±23.3	333.33 ±37.5	240.50 ±12.9	282.00 ±36.3	264.50 ±23.6	241.11 ±15.9
Revaccinated							
Control	225.50 ±20.2	280.50 ±21.5	286.00 ±27.78	290.00 ±31.4	249.00 ±24.0	269.50 ±20.8	294.00 ±21.9
VicS, eyedrop at day-old	235.56 ±23.5	285.79 ±36.4	261.50 ±21.3	249.00 ±25.6	236.00 ±15.9	297.50 ±44.7	227.00 ±10.8
VicS, coarse spray at day-old	202.67 ±10.2	270.67 ±37.1	268.57 ±27.3	324.00 ±45.6	231.00 ±19.2	278.50 ±39.4	249.00 ±21.1
VicS, in water at day-old	229.52 ±20.6	241.58 ±8.3	256.19 ±26.5	230.00 ±11.1	334.00 ±68.0	263.50 ±33.5	310.00 ±68.5
A3, eyedrop at day-old	244.29 ±17.38	312.35 ±35.3	288.00 ±37.1	242.00 ±16.8	239.00 ±11.9	253.50 ±23.2	243.00 ±22.9
A3, coarse spray at day-old	237.14 ±13.8	269.47 ±26.0	244.21 ±27.6	250.00 ±19.3	263.00 ±32.6	273.50 ±19.4	316.00 ±66.5
A3, in water at day-old	267.90 ±34.5	325.00 ±34.6	256.19 ±21.6	258.00 ±11.9	251.00 ±11.7	246.00 ±17.4	292.00 ±36.3

Values are Mean ± S.E.
62 wks, significant difference within shed (0.0114); 64 wks, no significant difference; 68 wks, no significant difference; 72 wks, no significant difference; 78 wks, no significant difference

Table D7 Egg quality shell weight (g) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		Moulting after revaccination	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	6.12 ±0.1	6.28 ±0.1	5.60 ±0.3	5.91 ±0.1	5.71 ±0.1	6.29 ±0.1	6.43 ±0.1
VicS, eyedrop at day-old	6.07 ±0.2	6.11 ±0.1	6.28 ±0.2	5.87 ±0.1	6.21 ±0.2	5.98 ±0.1	6.34 ±0.2
VicS, coarse spray at day-old	5.92 ±0.1	6.03 ±0.2	6.38 ±0.1	6.50 ±0.1	6.29 ±0.2	6.05 ±0.2	6.04 ±0.1
VicS, in water at day-old	6.11 ±0.2	6.13 ±0.2	6.26 ±0.1	6.10 ±0.1	5.94 ±0.2	6.02 ±0.2	6.21 ±0.2
A3, eyedrop at day-old	6.06 ±0.1	5.85 ±0.2	6.49 ±0.1	6.37 ±0.1	5.82 ±0.1	6.32 ±0.1	6.29 ±0.2
A3, coarse spray at day-old	6.11 ±0.1	5.97 ±0.2	6.46 ±0.1	6.37 ±0.1	6.08 ±0.3	6.16 ±0.1	6.26 ±0.3
A3, in water at day-old	6.07 ±0.2	6.04 ±0.1	6.26 ±0.1	6.21 ±0.1	6.54 ±0.2	6.28 ±0.1	6.80 ±0.2
Revaccinated							
Control	6.10 ±0.1	6.24 ±0.2	5.87 ±0.2	6.42 ±0.1	6.08 ±0.2	6.06 ±0.1	6.72 ±0.2
VicS, eyedrop at day-old	5.77 ±0.2	6.26 ±0.1	6.31 ±0.1	6.15 ±0.1	6.22 ±0.2	6.20 ±0.1	6.41 ±0.2
VicS, coarse spray at day-old	5.91 ±0.2	6.16 ±0.2	6.05 ±0.2	6.05 ±0.2	6.30 ±0.2	6.26 ±0.1	6.14 ±0.3
VicS, in water at day-old	6.12 ±0.2	6.18 ±0.2	6.16 ±0.2	5.98 ±0.1	6.65 ±0.1	6.12 ±0.2	6.50 ±0.3
A3, eyedrop at day-old	5.98 ±0.1	6.19 ±0.1	6.33 ±0.2	6.10 ±0.1	6.27 ±0.2	6.19 ±0.1	6.17 ±0.2
A3, coarse spray at day-old	6.01 ±0.1	6.12 ±0.1	6.47 ±0.1	6.14 ±0.1	6.00 ±0.2	6.10 ±0.1	6.06 ±0.2
A3, in water at day-old	5.99 ±0.1	6.12 ±0.2	6.52 ±0.2	6.34 ±0.2	6.39 ±0.2	6.12 ±0.1	6.31 ±0.2

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, no significant difference; 68 wks, significant difference within initial treatment (0.0001); 72 wks, interaction of shed, initial treatment and moulting (0.0037); 78 wks, significant difference initial treatment (0.0319)

Table D8 Egg quality percentage of egg shell (%) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		Moulting after revaccination	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	9.84 ±0.2	9.78 ±0.2	8.82 ±0.4	9.46 ±0.2	8.68 ±0.2	9.97 ±0.1	9.97 ±0.1
VicS, eyedrop at day-old	9.41 ±0.2	9.68 ±0.2	9.94 ±0.2	9.42 ±0.2	9.51 ±0.3	9.27 ±0.1	9.57 ±0.2
VicS, coarse spray at day-old	9.44 ±0.2	9.33 ±0.3	10.03 ±0.1	9.75 ±0.1	9.58 ±0.3	9.59 ±0.2	9.75 ±0.3
VicS, in water at day-old	9.81 ±0.3	9.80 ±0.2	9.91 ±0.2	9.58 ±0.2	9.22 ±0.3	9.17 ±0.2	9.74 ±0.2
A3, eyedrop at day-old	9.68 ±0.3	9.36 ±0.2	10.15 ±0.2	9.68 ±0.1	9.42 ±0.2	9.72 ±0.1	9.79 ±0.3
A3, coarse spray at day-old	9.44 ±0.2	9.13 ±0.3	9.93 ±0.2	9.58 ±0.2	8.96 ±0.4	9.30 ±0.1	9.75 ±0.2
A3, in water at day-old	9.73 ±0.4	9.56 ±0.1	10.17 ±0.2	9.67 ±0.2	10.17 ±0.2	9.62 ±0.2	10.33 ±0.2
Revaccinated							
Control	9.64 ±0.3	9.50 ±0.2	8.91 ±0.3	9.89 ±0.2	9.49 ±0.4	9.54 ±0.1	10.47 ±0.3
VicS, eyedrop at day-old	8.88 ±0.2	9.35 ±0.2	9.59 ±0.1	9.49 ±0.1	9.26 ±0.2	9.30 ±0.2	9.85 ±0.2
VicS, coarse spray at day-old	9.19 ±0.2	9.46 ±0.3	9.44 ±0.2	9.27 ±0.2	9.81 ±0.4	9.65 ±0.1	9.23 ±0.4
VicS, in water at day-old	9.68 ±0.3	9.60 ±0.2	9.43 ±0.2	9.23 ±0.1	9.65 ±0.2	9.53 ±0.2	10.03 ±0.3
A3, eyedrop at day-old	9.41 ±0.3	9.52 ±0.3	9.86 ±0.2	9.47 ±0.2	9.63 ±0.4	9.61 ±0.1	9.91 ±0.3
A3, coarse spray at day-old	10.07 ±0.3	9.41 ±0.2	9.85 ±0.1	9.50 ±0.2	9.34 ±0.4	9.72 ±0.2	9.52 ±0.3
A3, in water at day-old	9.45 ±0.2	9.52 ±0.2	10.08 ±0.1	9.60 ±0.2	9.73 ±0.2	9.58 ±0.2	9.66 ±0.2

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, no significant difference; 68 wks, significant difference within shed (0.0192), initial treatment (<0.0001); 72 wks, significant difference within initial treatment (0.0271), interaction of shed, initial treatment and moulting (0.0185); 78 wks, significant difference within moulting (0.0007), interaction between initial treatment and moulting (0.0435)

Table D9 Egg quality shell thickness (μm) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		**Moulting after revaccination**	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	437.52 ±5.2	433.89 ±9.0	418.16 ±12.0	417.10 ±6.9	393.80 ±5.5	439.70 ±4.7	430.90 ±4.4
VicS, eyedrop at day-old	417.30 ±6.6	426.06 ±6.1	436.05 ±6.8	418.65 ±7.4	428.10 ±9.7	416.10 ±5.4	434.80 ±9.4
VicS, coarse spray at day-old	417.65 ±9.0	418.95 ±9.0	444.19 ±4.0	442.40 ±5.9	430.40 ±11.8	421.90 ±6.9	429.80 ±6.5
VicS, in water at day-old	426.00 ±5.7	423.16 ±7.5	442.39 ±5.3	428.25 ±7.5	416.90 ±10.2	411.85 ±5.8	422.30 ±7.9
A3, eyedrop at day-old	433.00 ±5.8	409.71 ±9.0	446.29 ±6.4	436.55 ±6.2	417.20 ±6.4	434.05 ±5.9	446.50 ±13.5
A3, coarse spray at day-old	424.52 ±6.1	413.84 ±8.4	444.74 ±5.9	436.95 ±6.5	416.20 ±11.2	423.50 ±7.6	439.80 ±11.1
A3, in water at day-old	432.86 ±10.8	421.38 ±4.3	439.62 ±5.4	438.90 ±4.7	448.90 ±9.7	440.70 ±6.7	461.22 ±7.7
Revaccinated							
Control	419.76 ±8.2	422.35 ±7.2	406.90 ±10.8	449.35 ±7.9	422.00 ±12.8	426.70 ±6.0	468.30 ±13.4
VicS, eyedrop at day-old	410.68 ±9.4	423.32 ±5.6	436.14 ±5.2	427.80 ±5.4	434.10 ±7.6	431.00 ±6.1	442.20 ±6.6
VicS, coarse spray at day-old	411.80 ±8.6	423.69 ±8.5	427.48 ±8.7	416.30 ±9.1	433.90 ±13.6	425.75 ±5.0	424.80 11.77
VicS, in water at day-old	432.52 ±8.0	423.53 ±9.3	425.76 ±9.2	420.90 ±5.7	443.80 ±5.7	431.60 ±7.7	459.20 ±11.20
A3, eyedrop at day-old	425.10 ±5.4	434.37 ±7.5	445.95 ±7.4	430.20 ±6.2	438.90 ±9.4	426.75 ±5.0	447.90 ±13.9
A3, coarse spray at day-old	435.00 ±7.9	404.05 ±20.8	439.10 ±6.0	429.95 ±6.3	421.80 ±11.6	427.00 ±5.1	424.90 ±9.2
A3, in water at day-old	422.55 ±5.6	426.35 ±9.1	446.38 ±6.0	430.65 ±7.3	443.00 ±7.7	426.55 ±6.1	434.60 ±7.1

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, no significant difference; 68 wks, no significant difference; 72 wks, interaction between shed and initial treatment (0.0309), interaction of shed, initial treatment and moulting (0.0009); 78 wks, significant difference within shed (0.0202), moulting (0.0008), interaction between shed and initial treatment (0.0029), between initial treatment and moulting (0.0370)

Table D10 Egg quality albumen height (mm) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		**Moulting after revaccination**	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	7.34 ±0.3	7.62 ±0.4	7.99 ±0.4	8.07 ±0.3	7.06 ±0.5	8.92 ±0.2	8.16 ±0.3
VicS, eyedrop at day-old	6.99 ±0.3	7.92 ±0.4	7.52 ±0.4	7.05 ±0.3	7.69 ±0.7	8.40 ±0.3	7.64 ±0.6
VicS, coarse spray at day-old	6.43 ±0.3	8.47 ±0.3	7.69 ±0.3	7.95 ±0.3	7.62 ±0.4	8.75 ±0.4	7.59 ±0.5
VicS, in water at day-old	6.27 ±0.3	7.56 ±0.4	8.19 ±0.3	8.27 ±0.2	7.58 ±0.6	6.68 ±0.4	7.27 ±0.7
A3, eyedrop at day-old	6.66 ±0.3	8.77 ±0.2	8.31 ±0.3	8.03 ±0.3	7.23 ±0.6	7.53 ±0.4	7.76 ±0.5
A3, coarse spray at day-old	6.24 ±0.4	8.69 ±0.3	8.04 ±0.3	8.84 ±0.3	8.58 ±0.3	8.53 ±0.3	7.76 ±0.3
A3, in water at day-old	6.66 ±0.3	7.96 ±0.3	7.70 ±0.4	7.25 ±0.4	6.86 ±0.5	7.07 ±0.4	8.21 ±0.3
Revaccinated							
Control	6.75 ±0.3	8.54 ±0.3	7.64 ±0.3	8.10 ±0.3	7.42 ±0.4	8.22 ±0.3	7.78 ±0.5
VicS, eyedrop at day-old	6.74 ±0.4	8.98 ±0.3	8.40 ±0.3	8.56 ±0.3	7.80 ±0.7	8.72 ±0.4	8.63 ±0.3
VicS, coarse spray at day-old	6.77 ±0.5	7.91 ±0.4	7.40 ±0.3	7.34 ±0.5	6.42 ±0.7	8.44 ±0.4	7.79 ±0.6
VicS, in water at day-old	5.90 ±0.3	7.74 ±0.4	8.10 ±0.3	7.73 ±0.4	7.54 ±0.6	6.99 ±0.4	6.53 ±0.4
A3, eyedrop at day-old	6.91 ±0.3	7.86 ±0.3	7.61 ±0.2	8.03 ±0.2	7.06 ±0.3	8.74 ±0.3	8.07 ±0.5
A3, coarse spray at day-old	7.13 ±0.2	7.72 ±0.3	7.13 ±0.4	7.82 ±0.2	7.87 ±0.4	8.38 ±0.2	8.68 ±0.3
A3, in water at day-old	6.57 ±0.2	8.01 ±0.3	7.42 ±0.4	7.57 ±0.4	6.77 ±0.5	8.28 ±0.2	7.39 ±0.5

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, no significant difference; 68 wks, no significant difference; 72 wks, significant difference within initial treatment (<0.0001), interaction between shed and initial treatment (0.0024), between initial treatment and moulting (0.0005); 78 wks, significant difference within initial treatment (0.0440), moulting (0.0272)

Table D11 Egg quality Haugh unit (HU) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		Moulting after revaccination	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	84.33 ±1.7	84.78 ±2.7	87.42 ±2.2	88.65 ±1.7	80.80 ±3.6	93.25 ±1.1	88.80 ±1.6
VicS, eyedrop at day-old	81.00 ±2.1	86.89 ±2.3	84.15 ±2.8	81.90 ±2.2	84.60 ±3.9	90.00 ±1.6	83.70 ±4.6
VicS, coarse spray at day-old	77.15 ±2.8	90.19 ±1.7	85.95 ±2.0	86.40 ±2.0	84.50 ±3.2	92.05 ±1.9	85.70 ±3.0
VicS, in water at day-old	76.19 ±2.7	84.63 ±3.1	88.78 ±1.9	89.70 ±1.1	84.20 ±4.1	77.30 ±3.4	81.60 ±5.1
A3, eyedrop at day-old	79.05 ±2.3	92.71 ±1.0	89.48 ±1.6	87.55 ±1.5	82.40 ±4.7	84.00 ±2.5	86.10 ±2.8
A3, coarse spray at day-old	74.91 ±2.7	91.16 ±1.7	87.63 ±1.5	91.90 ±1.6	90.40 ±1.7	90.00 ±1.9	86.50 ±2.0
A3, in water at day-old	79.67 ±1.8	87.33 ±2.1	85.91 ±2.6	82.05 ±3.0	79.90 ±3.9	79.15 ±3.9	88.89 ±1.8
Revaccinated							
Control	79.68 ±2.3	90.30 ±1.3	85.35 ±1.5	87.75 ±1.9	84.10 ±2.5	88.95 ±1.9	86.00 ±3.1
VicS, eyedrop at day-old	78.11 ±3.4	92.32 ±1.7	88.95 ±2.2	90.50 ±1.8	83.90 ±5.4	90.05 ±2.9	91.20 ±1.9
VicS, coarse spray at day-old	78.53 ±3.9	86.44 ±2.5	83.52 ±2.7	81.80 ±3.3	74.50 ±6.3	88.85 ±3.0	85.10 ±3.6
VicS, in water at day-old	72.86 ±2.8	85.47 ±2.6	87.62 ±2.0	84.50 ±3.4	82.80 ±3.9	80.25 ±3.1	78.00 ±2.9
A3, eyedrop at day-old	80.95 ±1.8	86.21 ±2.3	85.65 ±1.3	87.95 ±1.4	81.70 ±1.9	91.60 ±1.8	88.40 ±3.0
A3, coarse spray at day-old	83.91 ±1.0	85.85 ±1.7	80.37 ±3.7	86.75 ±1.3	87.00 ±1.9	90.40 ±1.1	91.90 ±1.6
A3, in water at day-old	78.65 ±2.0	87.50 ±2.3	83.00 ±3.0	83.75 ±2.7	79.10 ±3.5	89.50 ±1.0	83.60 ±3.1

Values are Mean ± S.E.
62 wks, no significant difference; 64 wks, interaction between shed and initial treatment (0.0161); 68 wks, no significant difference; 72 wks, significant difference within initial treatment (<0.0001), interaction between shed and initial treatment (0.0057), between initial treatment and moulting (0.0005); 78 wks, significant difference within moulting (0.0137)

Table D12 Egg quality yolk color (Roche scale) of ISA brown hens following IB revaccination in different IB vaccination protocols during moulting phase

				Moulting before revaccination		Moulting after revaccination	
	Wk 62	Wk 64	Wk 68	Wk 72	Wk 78	Wk 72	Wk 78
Not revaccinated							
Control	11.38 ±0.2	11.11 ±0.2	10.11 ±0.2	11.40 ±0.1	9.00 ±0.4	11.15 ±0.2	10.20 ±0.3
VicS, eyedrop at day-old	11.75 ±0.1	11.22 ±0.3	10.25 ±0.1	11.00 ±0.2	9.20 ±0.3	11.35 ±0.2	10.20 ±0.2
VicS, coarse spray at day-old	11.25 ±0.2	11.76 ±0.2	10.43 ±0.3	10.90 ±0.2	10.30 ±0.3	10.70 ±0.3	10.00 ±0.4
VicS, in water at day-old	11.57 ±0.2	11.42 ±0.2	11.00 ±0.2	11.10 ±0.2	10.40 ±0.3	11.00 ±0.2	9.90 ±0.2
A3, eyedrop at day-old	11.33 ±0.2	11.57 ±0.2	10.52 ±0.2	10.95 ±0.1	9.40 ±0.2	10.65 ±0.2	10.10 ±0.4
A3, coarse spray at day-old	11.76 ±0.1	11.21 ±0.1	11.11 ±0.2	11.20 ±0.2	10.00 ±0.2	10.85 ±0.1	10.30 ±0.3
A3, in water at day-old	11.33 ±0.3	10.95 ±0.2	9.71 ±0.5	11.25 ±0.2	9.90 ±0.2	10.80 ±0.2	9.44 ±0.2
Revaccinated							
Control	11.33 ±0.2	11.25 ±0.1	10.30 ±0.2	11.30 ±0.2	9.70 ±0.3	10.25 ±0.2	11.30 ±0.2
VicS, eyedrop at day-old	11.58 ±0.2	11.11 ±0.1	10.95 ±0.2	10.55 ±0.2	10.40 ±0.2	10.20 ±0.2	10.70 ±0.3
VicS, coarse spray at day-old	11.33 ±0.2	12.00 ±0.1	10.43 ±0.5	11.00 ±0.2	10.70 ±0.3	10.90 ±0.2	10.50 ±0.3
VicS, in water at day-old	11.38 ±0.2	11.21 ±0.3	10.81 ±0.2	10.70 ±0.2	10.50 ±0.3	10.20 ±0.3	10.10 ±0.4
A3, eyedrop at day-old	11.38 ±0.2	11.16 ±0.1	11.0 ±0.2	11.10 ±0.2	10.30 ±0.2	10.40 ±0.2	10.20 ±0.4
A3, coarse spray at day-old	10.91 ±0.2	11.20 ±0.2	10.90 ±0.2	10.95 ±0.2	9.90 ±0.2	10.45 ±0.2	10.10 ±0.4
A3, in water at day-old	10.90 ±0.2	11.05 ±0.2	10.14 ±0.3	10.40 ±0.1	10.60 ±0.3	10.85 ±0.2	10.30 ±0.3

Values are Mean ± S.E.
62 wks, significant difference within shed (0.0323); 64 wks, significant difference within initial treatment (0.0001); 68 wks, significant difference within initial treatment(0.0007); 72 wks, significant difference within shed (<0.0001), moulting (<0.0001), interaction between shed and initial treatment (0.0095), interaction of shed, initial treatment and moulting (0.0367); 78 wks, significant difference within shed (<0.0001), moulting (0.0479), between initial treatment and moulting (<0.0001)

Table D13 Excreta moisture (%) after revaccination of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Moulting before vaccination		**Moulting after vaccination**	
	Wk 1	Wk 2	Wk 1	Wk 2
Not revaccinated	71.28 ±1.2	66.59 ±1.1	82.22 ±2.1	80.36 ±1.8
Control	76.92 ±3.9	73.36 ±2.8	82.74 ±3.7	86.78 ±1.5
VicS, eyedrop at day-old	69.65 ±1.6	66.36 ±3.2	84.14 ±2.8	80.82 ±4.7
VicS, coarse spray at day-old	76.48 ±0.9	67.16 ±1.8	79.41 ±9.0	91.19 ±3.7
VicS, in water at day-old	69.70 ±3.7	64.45 ±2.3	74.37 ±10.3	80.99 ±4.2
A3, eyedrop at day-old	67.20 ±2.5	64.47 ±3.8	84.17 ±3.3	74.85 ±2.6
A3, coarse spray at day-old	68.66 ±2.6	64.25 ±3.1	84.27 ±4.3	74.69 ±4.1
A3, in water at day-old	70.03 ±4.2	66.05 ±1.9	86.05 ±2.8	76.26 ±6.0
Revaccinated	72.65 ±1.0	68.93 ±1.1	85.58 ±1.6	86.88 ±1.1
Control	75.30 ±1.1	74.56 ±2.4	84.22 ±2.2	87.40 ±2.0
VicS, eyedrop at day-old	69.85 ±3.7	69.99 ±1.2	93.65 ±2.7	87.12 ±1.1
VicS, coarse spray at day-old	75.82 ±3.3	71.21 ±4.2	90.97 ±2.1	86.79 ±4.2
VicS, in water at day-old	69.72 ±2.6	66.74 ±2.4	85.07 ±3.9	88.46 ±2.9
A3, eyedrop at day-old	73.21 ±2.1	69.13 ±1.2	81.69 ±4.7	87.16 ±2.3
A3, coarse spray at day-old	71.66 ±1.5	68.77 ±3.0	88.31 ±4.8	87.53 ±6.6
A3, in water at day-old	72.41 ±3.8	63.67 ±3.0	80.53 ±5.4	84.05 ±3.5

Values are Mean ± S.E.
Significant difference within sheds (0.0015), initial treatments (0.0223), moulting (<0.0001)

Table D14 Haematocrit value (%) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Moulting before vaccination			Moulting after vaccination		
	Wk 58	Wk 65	Wk 77	Wk 58	Wk 65	Wk 77
Not revaccinated	35.31 ±0.8	28.94 ±0.9	29.75 ±0.6	30.97 ±0.7	34.00 ±0.8	29.08 ±0.6
Control	36.17 ±1.2	28.00 ±3.0	29.17 ±2.9	33.50 ±1.5	35.25 ±0.25	30.50 ±0.5
VicS, eyedrop at day-old	32.67 ±1.7	30.83 ±2.6	29.83 ±0.6	30.25 ±4.2	33.50 ±2.5	32.00 ±3.0
VicS, coarse spray at day-old	32.67 ±1.7	31.17 ±2.0	30.67 ±1.4	31.50 ±0.0	30.75 ±3.7	30.00 ±1.00
VicS, in water at day-old	36.50 ±0.0	29.00 ±1.0	30.00 ±0.0	31.33 ±2.2	38.00 ±1.8	29.67 ±0.9
A3, eyedrop at day-old	36.50 ±3.5	28.50 ±0.5	30.00 ±0.0	30.33 ±0.9	33.50 ±0.9	29.17 ±1.4
A3, coarse spray at day-old	38.75 ±3.7	24.50 ±1.5	29.50 ±0.5	29.17 ±0.2	35.67 ±1.1	26.33 ±0.6
A3, in water at day-old	37.00 ±0.0	29.00 ±0.0	28.00 ±0.0	31.37 ±2.4	31.37 ±1.2	28.00 ±1.3
Revaccinated	34.10 ±0.5	28.07 ±0.9	29.17 ±0.6	32.50 ±0.8	32.60 ±0.8	29.17 ±0.5
Control	36.00 ±1.0	36.00 ±6.0	28.75 ±2.7	31.33 ±1.9	31.17 ±1.6	28.67 ±1.2
VicS, eyedrop at day-old	31.67 ±2.0	25.83 ±1.4	28.33 ±1.0	32.75 ±2.2	31.25 ±0.2	29.50 ±0.5
VicS, coarse spray at day-old	36.25 ±2.7	28.00 ±0.0	32.25 ±1.7	34.33 ±2.2	30.83 ±2.7	30.83 ±0.7
VicS, in water at day-old	35.50 ±0.3	28.00 ±1.3	31.17 ±1.6	33.50 ±0.5	34.00 ±4.0	28.00 ±1.0
A3, eyedrop at day-old	33.50 ±0.5	27.17 ±1.4	28.50 ±1.3	33.50 ±3.5	35.50 ±2.5	29.00 ±1.0
A3, coarse spray at day-old	32.50 ±0.5	28.33 ±1.4	31.00 ±0.0	30.75 ±3.2	34.25 ±0.2	30.25 ±1.2
A3, in water at day-old	34.50 ±0.5	26.37 ±1.0	26.12 ±1.0	29.50 ±0.0	33.00 ±0.0	25.50 ±0.0

Values are Mean ± S.E.
Significant difference within ages (<.0001);

Table D15 Plasma Sodium (Na^+, mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Moulting before vaccination			**Moulting after vaccination**		
	Wk 58	Wk 65	Wk 77	Wk 58	Wk 65	Wk 77
Not revaccinated	145.3 ±0.9	149.6 ±0.6	155.6 ±0.6	146.3 ±0.7	147.5 ±1.0	152.6 ±0.9
Control	145.4 ±0.1	149.6 ±1.8	157.0 ±1.4	149.1 ±1.3	146.1 ±0.0	157.7 ±2.9
VicS, eyedrop at day-old	145.9 ±2.4	150.6 ±0.3	155.3 ±1.9	148.7 ±0.9	149.2 ±0.7	157.1 ±0.1
VicS, coarse spray at day-old	149.9 ±2.4	150.2 ±1.6	156.6 ±2.2	148.1 ±1.7	153.9 ±0.2	154.7 ±1.4
VicS, in water at day-old	142.3 ±2.6	148.0 ±1.1	155.8 ±1.3	144.1 ±2.2	144.9 ±0.5	150.7 ±1.6
A3, eyedrop at day-old	145.8 ±0.8	151.5 ±1.7	155.9 ±0.8	145.3 ±0.2	151.2 ±0.9	149.2 ±1.8
A3, coarse spray at day-old	140.9 ±2.6	147.4 ±0.9	154.1 ±0.2	144.4 ±3.2	146.5 ±1.7	149.0 ±1.0
A3, in water at day-old	144.2 ±1.4	149.7 ±3.3	153.8 ±0.0	146.6 ±0.6	143.1 ±3.2	153.9 ±1.7
Revaccinated	144.3 ±0.7	150.2 ±0.5	154.4 ±0.8	145.9 ±0.8	148.1 ±0.7	154.99 ±1.0
Control	141.7 ±0.4	150.5 ±0.6	149.3 ±1.9	146.6 ±1.6	148.5 ±1.2	157.0 ±1.5
VicS, eyedrop at day-old	145.3 ±2.9	150.2 ±1.4	154.9 ±1.4	143.7 ±2.4	145.1 ±0.1	155.3 ±0.6
VicS, coarse spray at day-old	145.2 ±0.6	152.1 ±0.2	158.6 ±2.1	146.1 ±0.8	149.6 ±2.0	156.97 ±0.7
VicS, in water at day-old	146.6 ±0.9	150.9 ±1.4	157.4 ±1.4	144.4 ±5.7	148.0 ±4.2	152.10 ±6.2
A3, eyedrop at day-old	141.6 ±0.8	150.8 ±1.2	152.3 ±1.6	143.6 ±2.5	147.8 ±1.7	151.0 ±3.0
A3, coarse spray at day-old	144.9 ±0.4	150.7 ±1.1	155.5 ±0.5	147.4 ±1.0	146.9 ±0.2	151.4 ±0.8
A3, in water at day-old	144.2 ±2.6	146.7 ±1.3	152.5 ±1.1	149.4 ±1.1	149.6 ±2.6	159.2 ±0.4

Values are Mean ± S.E.
Significant difference within initial treatments (0.0139); within ages (<.0001); Interaction between shed and initial treatment (0.0445);

Table D16 Plasma Potassium (K^+, mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Moulting before vaccination			Moulting after vaccination		
	Wk 58	Wk 65	Wk 77	Wk 58	Wk 65	Wk 77
Not revaccinated	4.37 ±0.1	5.15 ±0.1	5.60 ±0.1	4.85 ±0.1	4.98 ±0.1	5.53 ±0.1
Control	4.01 ±0.5	5.33 ±0.1	5.31 ±0.4	5.17 ±0.1	5.05 ±0.5	5.72 ±0.5
VicS, eyedrop at day-old	4.52 ±0.3	5.29 ±0.1	5.59 ±0.1	5.07 ±0.5	5.11 ±0.1	5.91 ±0.1
VicS, coarse spray at day-old	4.87 ±0.2	4.72 ±0.2	5.50 ±0.1	4.33 ±0.4	5.67 ±0.05	5.51 ±0.4
VicS, in water at day-old	4.10 ±0.5	5.04 ±0.3	5.46 ±0.2	5.17 ±0.3	5.19 ±0.6	5.15 ±0.3
A3, eyedrop at day-old	4.70 ±0.5	5.28 ±0.2	5.74 ±0.1	4.69 ±0.2	5.21 ±0.5	5.88 ±0.2
A3, coarse spray at day-old	4.04 ±0.1	5.03 ±0.2	5.76 ±0.2	4.72 ±0.1	4.38 ±0.02	5.19 ±0.03
A3, in water at day-old	4.19 ±0.5	5.40 ±0.2	6.07 ±0.3	4.78 ±0.2	4.55 ±0.1	5.55 ±0.4
Revaccinated	4.46 ±0.11	5.31 ±0.07	0.36 ±0.08	4.78 ±0.12	4.89 ±0.15	5.51 ±0.11
Control	4.09 ±0.1	5.00 ±0.2	5.18 ±0.3	4.86 ±0.1	4.68 ±0.1	5.39 ±0.2
VicS, eyedrop at day-old	4.43 ±0.6	5.25 ±0.3	5.57 ±0.3	4.74 ±0.3	4.35 ±0.1	5.48 ±0.1
VicS, coarse spray at day-old	4.45 ±0.04	5.27 ±0.1	5.27 ±0.1	4.41 ±0.3	5.31 ±0.3	5.67 ±0.2
VicS, in water at day-old	4.78 ±0.04	5.40 ±0.2	5.80 ±0.1	4.56 ±0.9	4.45 ±0.9	5.34 ±0.9
A3, eyedrop at day-old	4.30 ±0.2	5.35 ±0.2	5.45 ±0.1	4.73 ±0.3	5.27 ±0.4	5.52 ±0.4
A3, coarse spray at day-old	4.43 ±0.2	5.46 ±0.1	5.62 ±0.1	5.15 ±0.2	4.92 ±0.4	5.22 ±0.04
A3, in water at day-old	4.60 ±0.4	5.33 ±0.2	5.49 ±0.4	5.20 ±0.1	5.14 ±0.4	5.97 ±0.1

Values are Mean ± S.E.
Significant difference within ages (<0.0001); Interaction between age and moulting (<0.0001)

Table D17 Plasma Calcium (Ca^{++}, mmol/L) of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Moulting before vaccination			**Moulting after vaccination**		
	Wk 58	Wk 65	Wk 77	Wk 58	Wk 65	Wk 77
Non revaccinated	1.38 ±0.04	1.76 ±0.05	1.80 ±0.03	1.55 ±0.04	1.83 ±0.06	1.78 ±0.02
Control	1.39 ±0.10	1.83 ±0.07	1.76 ±0.04	1.60 ±0.01	2.10 ±0.04	1.82 ±0.01
VicS, eyedrop at day-old	1.35 ±0.08	1.68 ±0.11	1.76 ±0.07	1.74 ±0.05	2.09 ±0.01	1.77 ±0.00
VicS, coarse spray at day-old	1.55 ±0.09	1.86 ±0.25	1.94 ±0.06	1.36 ±0.01	2.07 ±0.16	1.84 ±0.01
VicS, in water at day-old	1.23 ±0.06	1.81 ±0.03	1.75 ±0.03	1.60 ±0.2	1.63 ±0.19	1.71 ±0.09
A3, eyedrop at day-old	1.50 ±0.2	1.77 ±0.02	1.85 ±0.06	1.43 ±0.03	1.68 ±0.11	1.78 ±0.06
A3, coarse spray at day-old	1.21 ±0.13	1.79 ±0.10	1.79 ±0.07	1.55 ±0.11	1.78 ±0.05	1.77 ±0.08
A3, in water at day-old	1.37 ±0.13	1.57 ±0.01	1.71 ±0.16	1.56 ±0.05	1.73 ±0.19	1.83 ±0.02
Revaccinated	1.34 ±0.04	1.78 ±0.03	1.79 ±0.02	1.50 ±0.04	1.73 ±0.08	1.79 ±0.03
Control	1.20 ±0.02	1.56 ±0.09	1.67 ±0.04	1.57 ±0.03	1.59 ±0.09	1.75 ±0.10
VicS, eyedrop at day-old	1.33 ±0.06	1.75 ±0.08	1.74 ±0.02	1.45 ±0.16	1.86 ±0.36	1.79 ±0.05
VicS, coarse spray at day-old	1.36 ±0.15	1.90 ±0.04	1.93 ±0.02	1.35 ±0.05	1.87 ±0.29	1.82 ±0.06
VicS, in water at day-old	1.56 ±0.07	1.87 ±0.08	1.81 ±0.02	1.41 ±0.19	1.55 ±0.04	1.74 ±0.07
A3, eyedrop at day-old	1.20 ±0.07	1.72 ±0.07	1.81 ±0.03	1.52 ±0.16	1.58 ±0.09	1.81 ±0.04
A3, coarse spray at day-old	1.34 ±0.09	1.81 ±0.03	1.78 ±0.07	1.57 ±0.13	1.82 ±0.32	1.77 ±0.09
A3, in water at day-old	1.36 ±0.04	1.84 ±0.06	1.78 ±0.01	1.59 ±0.03	1.85 ±0.19	1.87 ±0.09

Values are Mean ± S.E.
Significant difference of moulting (0.0186) and within ages (<0.0001)

Table D18 IBV antibody titres of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Moulting before vaccination			Moulting after vaccination		
	Wk 58	Wk 65	Wk 77	Wk 58	Wk 65	Wk 77
Not revaccinated	450 ±171	621 ±248	816.62 ±283.5	401 ±140	576.9 ±149	301.8 ±49.3
Control	670.0 ±457	747.67 ±519.2	753.33 ±477.4	1400.5 ±1181	1274 ±1041	247.0 ±83
VicS, eyedrop at day-old	125.7 ±66.2	743.33 ±723.9	1490.3 ±1130.6	69.50 ±68.5	215.0 ±73.0	255.5 ±123
VicS, coarse spray at day-old	982.7 ±819	1244.7 ±1119	1170.0 ±997	147.00 ±9.0	136.0 ±62.0	118.5 ±59.5
VicS, in water at day-old	232.5 ±198	198.50 ±174.5	200.50 ±170.5	52.67 ±5.4	204.3 ±132	159.7 ±48.4
A3, eyedrop at day-old	398.0 ±230	359.00 ±246.0	810.00 ±620.0	519.33 ±169.4	767.0 ±218	427.0 ±168
A3, coarse spray at day-old	160.0 ±22.0	91.00 ±22.0	280.00 ±134.0	642.33 ±313.5	632.3 ±239	386.7 ±127
A3, in water at day-old	288.0 ±0.0	438.00 ±0.0	244.00 ±0.0	186.75 ±54.9	724.8 ±454	393.0 ±147
Revaccinated	323.7 ±80.9	281.50 ±66.1	324.35 ±69.0	347.53 ±107.0	836.5 ±219	523.7 ±148
Control	450.0 ±268	269.00 ±97.0	211.00 ±39.0	210.67 ±68.2	1129. ±517	273.3 ±58.5
VicS, eyedrop at day-old	85.33 ±41.1	90.33 ±37.7	120.33 ±50.3	87.00 ±63.0	165.5 ±37.5	139.5 ±44.5
VicS, coarse spray at day-old	566.0 ±410	479.50 ±341.5	517.50 ±333.5	745.33 ±388.	794.7 ±309	768.3 ±306
VicS, in water at day-old	154.7 ±106	116.3 ±86.3	84.67 ±30.2	77.50 ±8.5	76.50 ±20.5	65.50 ±6.5
A3, eyedrop at day-old	256.0 ±82.3	318.67 ±166.7	406.67 ±194.0	318.00 ±74.0	990.5 ±734	540.5 ±236
A3, coarse spray at day-old	282.7 ±91.8	246.33 ±69.3	388.33 ±111.3	602.00 ±488.0	779.0 ±641	584.5 ±458
A3, in water at day-old	526.5 ±322	454.50 ±251.9	507.50 ±241.2	176.00 ±0.0	2754. ±0.0	2070. ±0.0

Values are Mean ± S.E.
Significant difference within ages (0.0023)

Table D19 IBV antibody titres of ISA brown hens exposed to the following different IB vaccination protocols during moulting phase

	Wk 58	Wk 65	Wk 77	All ages
Not revaccinated	423.66 ±107.8	597.23 ±137.4	537.14 ±137.3	519.34 ±73.7
Control	962.20 ±484.0	958.40 ±453.9	550.80 ±290.6	823.80 ±229.4
VicS, eyedrop at day-old	103.20 ±44.4	532.00 ±417.7	996.40 ±690.3	543.87 ±267.7
VicS, coarse spray at day-old	648.40 ±493.2	801.20 ±671.0	749.40 ±604.4	733.00 ±318.0
VicS, in water at day-old	124.60 ±76.7	202.00 ±91.3	176.00 ±60.9	167.53 ±42.2
A3, eyedrop at day-old	470.80 ±121.6	603.80 ±174.3	580.20 ±236.1	551.60 ±99.2
A3, coarse spray at day-old	449.40 ±208.6	415.80 ±186.5	344.00 ±85.9	403.07 ±91.1
A3, in water at day-old	207.00 ±47.1	667.40 ±356.8	363.20 ±117.8	412.53 ±127.5
Revaccinated	333.91 ±64.1	519.37 ±110.1	409.77 ±75.3	421.02 ±49.4
Control	306.40 ±109.6	785.00 ±354.5	248.40 ±37.6	446.60 ±131.8
VicS, eyedrop at day-old	86.00 ±30.1	120.40 ±30.1	128.00 ±31.3	111.47 ±17.0
VicS, coarse spray at day-old	673.60 ±253.1	668.60 ±215.1	668.00 ±207.6	670.07 ±120.9
VicS, in water at day-old	123.80 ±61.4	100.40 ±48.7	77.00 ±17.3	100.40 ±25.3
A3, eyedrop at day-old	280.80 ±53.0	587.40 ±298.9	460.20 ±134.0	442.80 ±107.8
A3, coarse spray at day-old	410.40 ±180.2	459.40 ±244.0	466.80 ±164.5	445.53 ±106.7
A3, in water at day-old	456.40 ±259.5	914.40 ±499.6	820.00 ±364.1	730.27 ±213.5
All groups	378.79^{b} ±62.5	558.30^{a} ±87.5	473.46^{ab} ±78.1	

Values are Mean ± S.E.
Significant difference within ages (0.0023)

Table D20 Body weight (BW) (g) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Time Over TRTs
0	2112.84 ±40.1	2088.08 ±45.4	2066.16 ±49.9	2134.21 ±50.4	2133.42 ±28.0	2163.59 ±56.7	2070.92 ±37.7	2109.89 ±16.7
1	1970.72 ±34.0	2090.69 ±56.2	1901.06 ±37.4	2093.09 ±68.1	2159.25 ±95.0	2051.45 ±62.7	2103.69 ±70.0	2052.85 ±25.2
2	2093.15 ±100.8	2190.06 ±113.5	2046.27 ±74.4	2026.26 ±51.6	2143.02 ±52.5	2176.01 ±91.4	2139.20 ±60.5	2116.28 ±30.0
3	2194.89 ±63.9	2090.61 ±58.0	2062.29 ±84.1	2043.06 ±56.1	2092.32 ±85.3	2062.90 ±92.6	2252.60 ±82.7	2114.10 ±28.8
4	2158.72 ±44.4	1979.84 ±113.2	2148.36 ±78.7	2200.60 ±115.0	2235.60 ±66.2	2198.46 ±76.2	2173.80 ±39.3	2156.48 ±30.8
5	2181.72 ±92.4	2190.58 ±126.6	2192.92 ±91.7	2214.28 ±48.3	2089.90 ±82.8	1992.38 ±52.0	2327.22 ±107.7	2169.86 ±35.0
TRTs Over time	2111.44 ±27.3	2106.09 ±32.0	2056.03 ±29.0	2108.35 ±26.3	2138.81 ±26.7	2113.92 ±31.0	2159.13 ±28.2	

No significant difference within initial treatments and weeks
TRTs = treatments

Table D21 Total kidney weight (g) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Over TRTs
0	14.41 ±0.58	14.74 ±0.51	14.22 ±0.40	13.99 ±0.41	15.19 ±0.29	15.10 ±0.37	15.65 ±0.36	14.76 ±0.17
1	12.70 ±0.40	13.85 ±0.48	12.82 ±0.23	12.17 ±0.43	13.85 ±0.56	12.77 ±1.92	14.12 ±0.67	13.18 ±0.32
2	12.83 ±0.57	11.68 ±1.75	13.07 ±0.65	13.76 ±0.60	13.31 ±0.75	14.12 ±0.59	13.19 ±0.72	13.13 ±0.33
3	13.33 ±0.47	13.88 ±0.67	13.37 ±0.54	14.12 ±0.70	13.73 ±0.44	12.99 ±0.48	13.96 ±0.69	13.63 ±0.21
4	13.03 ±0.89	11.77 ±1.15	12.10 ±0.68	14.13 ±0.51	13.91 ±0.51	14.14 ±0.61	15.99 ±1.23	13.58 ±0.37
5	14.28 ±0.69	13.49 ±0.94	14.28 ±0.82	14.32 ±0.93	14.12 ±0.87	14.81 ±0.54	15.24 ±0.88	14.36 ±0.29
Over time	13.48 ±0.25	13.45 ±0.41	13.39 ±0.23	13.71 ±0.25	14.10 ±0.23	14.02 ±0.39	14.65 ±0.30	

Significant difference within weeks (< .0001) and initial treatments (0.011)
TRTs = treatments

Table D22 Percentages Total kidneys of BW (%) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Over TRTs
0	0.68 ±0.03	0.71 ±0.02	0.69 ±0.03	0.66 ±0.03	0.71 ±0.02	0.70 ±0.01	0.76 ±0.02	0.70 ±0.01
1	0.64 ±0.01	0.67 ±0.03	0.68 ±0.02	0.58 ±0.02	0.65 ±0.03	0.62 ±0.10	0.67 ±0.03	0.64 ±0.02
2	0.61 ±0.02	0.53 ±0.08	0.64 ±0.02	0.68 ±0.04	0.62 ±0.02	0.65 ±0.02	0.62 ±0.03	0.62 ±0.01
3	0.61 ±0.03	0.66 ±0.03	0.65 ±0.03	0.70 ±0.04	0.66 ±0.04	0.64 ±0.03	0.62 ±0.03	0.65 ±0.01
4	0.60 ±0.04	0.60 ±0.70	0.56 ±0.02	0.65 ±0.03	0.62 ±0.03	0.65 ±0.03	0.73 ±0.05	0.63 ±0.02
5	0.65 ±0.02	0.62 ±0.05	0.65 ±0.02	0.65 ±0.05	0.68 ±0.05	0.74 ±0.03	0.66 ±0.03	0.66 ±0.01
Over time	0.64 ±0.01	0.64 ±0.02	0.65 ±0.01	0.65 ±0.01	0.66 ±0.01	0.67 ±0.02	0.68 ±0.01	

Significant difference within weeks (< .0001)
TRTs = treatments

Table D23 Right kidneys weight (g) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Over TRTs
0	7.08 ±0.29	7.43 ±0.29	7.14 ±0.23	7.04 ±0.22	7.52 ±0.16	7.58 ±0.22	7.85 ±0.22	7.38 ±0.09
1	6.14 ±0.15	6.81 ±0.27	6.37 ±0.15	6.07 ±0.18	6.91 ±0.29	7.24 ±0.35	7.07 ±0.32	6.65 ±0.11
2	6.44 ±0.34	7.48 ±0.29	6.51 ±0.50	6.81 ±0.32	6.59 ±0.40	6.91 ±0.29	6.59 ±0.36	6.62 ±0.13
3	6.51 ±0.20	6.86 ±0.34	6.75 ±0.27	6.95 ±0.36	6.77 ±0.24	6.48 ±0.26	6.94 ±0.40	6.75 ±0.11
4	6.37 ±0.43	6.28 ±0.60	5.98 ±0.49	6.98 ±0.25	6.85 ±0.33	6.93 ±0.31	7.86 ±0.52	6.75 ±0.18
5	7.18 ±0.43	6.60 ±0.42	7.11 ±0.36	7.07 ±0.58	6.94 ±0.52	7.26 ±0.27	7.55 ±0.47	7.10 ±0.16
Over time	6.64 ±0.13	6.85 ±0.15	6.69 ±0.14	6.82 ±0.13	6.97 ±0.13	7.11 ±0.12	7.31 ±0.15	

Significant difference within weeks (< .0001) and initial treatments (.0037)
TRTs = treatments

Table D24 Percentages right kidneys of BW (%) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Over TRTs
0	0.34 ±0.01	0.36 ±0.01	0.32 ±0.03	0.33 ±0.01	0.32 ±0.03	0.35 ±0.01	0.38 ±0.01	0.34 ±0.01
1	0.31 ±0.01	0.33 ±0.02	0.33 ±0.01	0.29 ±0.01	0.32 ±0.01	0.31 ±0.05	0.34 ±0.01	0.32 ±0.01
2	0.31 ±0.01	0.25 ±0.04	0.32 ±0.02	0.34 ±0.02	0.31 ±0.01	0.32 ±0.01	0.31 ±0.02	0.31 ±0.01
3	0.30 ±0.01	0.33 ±0.01	0.33 ±0.01	0.34 ±0.02	0.33 ±0.02	0.32 ±0.01	0.31 ±0.02	0.32 ±0.01
4	0.30 ±0.02	0.32 ±0.03	0.28 ±0.02	0.32 ±0.02	0.31 ±0.02	0.32 ±0.02	0.36 ±0.02	0.31 ±0.01
5	0.33 ±0.01	0.30 ±0.02	0.32 ±0.01	0.32 ±0.03	0.33 ±0.03	0.36 ±0.01	0.32 ±0.02	0.33 ±0.01
Over time	0.31 ±0.01	0.32 ±0.01	0.32 ±0.01	0.32 ±0.01	0.32 ±0.01	0.33 ±0.01	0.34 ±0.01	

Significant difference within weeks (.013)
TRTs = treatments

Table D25 Left kidneys weight (g) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Over TRTs
0	7.34 ±0.33	7.32 ±0.25	7.08 ±0.22	6.94 ±0.23	7.67 ±0.16	7.52 ±0.21	7.79 ±0.18	7.38 ±0.09
1	6.56 ±0.25	7.04 ±0.26	6.46 ±0.15	6.10 ±0.27	6.94 ±0.30	7.36 ±0.37	7.04 ±0.37	6.78 ±0.11
2	6.38 ±0.25	6.86 ±0.34	6.56 ±0.20	6.95 ±0.30	6.72 ±0.35	7.21 ±0.31	6.59 ±0.38	6.75 ±0.12
3	6.82 ±0.27	7.02 ±0.34	6.62 ±0.29	7.16 ±0.36	6.96 ±0.21	6.51 ±0.25	7.02 ±0.31	6.87 ±0.11
4	6.66 ±0.46	5.49 ±0.83	6.12 ±0.21	7.15 ±0.28	7.06 ±0.19	7.21 ±0.34	8.14 ±0.71	6.83 ±0.21
5	7.10 ±0.28	6.89 ±0.52	7.17 ±0.47	7.25 ±0.40	7.19 ±0.36	7.55 ±0.30	7.69 ±0.43	7.26 ±0.14
Over time	6.85 ±0.13	6.89 ±0.17	6.70 ±0.11	6.89 ±0.13	7.12 ±0.11	7.23 ±0.12	7.35 ±0.16	

Significant difference within weeks (< .0001) and initial treatments (.0012)
TRTs = treatments

Table D26 Percentages left kidneys of BW (%) of treated chickens on every week post exposed to T strain IBV

Week	Initial Vaccine Treatments							
	Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water	Over TRTs
0	0.35 ±0.01	0.35 ±0.01	0.32 ±0.03	0.33 ±0.01	0.33 ±0.03	0.35 ±0.01	0.38 ±0.01	0.34 ±0.01
1	0.33 ±0.01	0.34 ±0.02	0.34 ±0.01	0.29 ±0.01	0.33 ±0.02	0.31 ±0.05	0.34 ±0.02	0.33 ±0.01
2	0.31 ±0.01	0.27 ±0.04	0.32 ±0.01	0.34 ±0.02	0.31 ±0.01	0.33 ±0.01	0.31 ±0.02	0.31 ±0.01
3	0.31 ±0.01	0.34 ±0.01	0.32 ±0.02	0.35 ±0.02	0.34 ±0.02	0.32 ±0.01	0.31 ±0.01	0.33 ±0.01
4	0.31 ±0.02	0.29 ±0.05	0.29 ±0.01	0.33 ±0.01	0.32 ±0.01	0.33 ±0.02	0.37 ±0.03	0.32 ±0.01
5	0.33 ±0.01	0.32 ±0.03	0.33 ±0.01	0.33 ±0.02	0.35 ±0.02	0.38 ±0.01	0.33 ±0.01	0.34 ±0.01
Over time	0.32 ±0.01	0.32 ±0.01	0.32 ±0.01	0.33 ±0.01	0.33 ±0.01	0.34 ±0.01	0.34 ±0.01	

No significant difference within initial treatments and weeks
TRTs = treatments

Appendix E

Table E1 The effect of challenge on egg shell quality.

		Before	1 Wk	2 Wks	3 Wks	4 Wks	5 Wks	P Value
Deformation (μm)	Control	275.9^{a} ±3.8	275.3^{a} ±5.2	261.2^{a} ±3.3	260.3^{b} ±3.2	266.3^{a} ±3.0	251.6^{b} ±2.1	0.0040
	T	274.8^{a} ±6.7	257.0^{b} ±3.6	268.4^{a} ±7.3	254.5^{b} ±3.9	264.9^{a} ±3.2	267.1^{a} ±5.1	
	N1/88	274.6^{a} ±6.5	277.4^{a} ±7.3	262.3^{a} ±4.6	275.6^{a} ±7.3	261.9^{a} ±3.7	268.7^{a} ±4.2	
Breaking Strength (N)	Control	40.28^{a} ±0.42	40.97^{b} ±0.38	42.01^{a} ±0.40	41.97^{a} ±0.39	44.27^{a} ±1.33	42.46^{a} ±0.37	0.1983
	T	41.43^{a} ±0.55	43.24^{a} ±0.57	42.75^{a} ±0.64	40.75^{a} ±0.54	43.33^{a} ±0.50	41.33^{a} ±0.58	
	N1/88	40.50^{a} ±0.65	42.37^{a} ±0.61	42.53^{a} ±0.52	41.14^{a} ±0.54	42.96^{a} ±0.54	41.81^{a} ±0.53	
Reflectivity (%)	Control	40.99^{a} ±0.70	41.44^{a} ±0.72	41.83^{a} ±0.74	41.40^{a} ±0.73	42.93^{a} ±0.72	43.87^{a} ±0.74	0.0006
	T	40.73^{a} ±1.02	43.59^{a} ±1.09	41.87^{a} ±1.11	42.11^{a} ±1.10	41.94^{a} ±1.04	41.90^{a} ±1.04	
	N1/88	40.23^{a} ±1.00	42.47^{a} ±1.06	41.98^{a} ±0.98	42.09^{a} ±1.11	42.32^{a} ±1.04	41.91^{a} ±1.03	
Egg Weight (g)	Control	61.27^{a} ±0.36	62.68^{a} ±0.26	62.76^{a} ±0.26	63.01^{a} ±0.24	63.30^{a} ±0.27	62.99^{a} ±0.25	<0.0001
	T	62.34^{a} ±0.34	58.87^{b} ±0.83	60.36^{b} ±0.62	57.55^{b} ±1.10	59.42^{b} ±0.92	59.51^{b} ±0.92	
	N1/88	61.50^{a} ±0.63	55.71^{c} ±1.51	61.96^{a} ±0.34	59.15^{b} ±1.05	59.51^{b} ±0.99	61.98^{a} ±0.50	
Shell Weight (g)	Control	5.75^{a} ±0.04	5.98^{a} ±0.03	6.29^{a} ±0.03	6.13^{a} ±0.03	6.15^{a} ±0.03	6.11^{a} ±0.03	<0.0001
	T	5.83^{a} ±0.05	5.95^{a} ±0.05	6.20^{ab} ±0.05	5.91^{b} ±0.05	5.95^{b} ±0.05	5.85^{b} ±0.05	
	N1/88	5.81^{a} ±0.05	5.96^{a} ±0.05	6.15^{b} ±0.05	5.97^{b} ±0.04	6.01^{b} ±0.04	5.92^{b} ±0.04	
% Shell	Control	415.5^{a} ±2.26	426.5^{b} ±1.94	431.8^{a} ±1.76	425.8^{a} ±1.81	425.1^{a} ±1.80	425.18^{a} ±1.80	0.0609
	T	417.5^{a} ±2.83	432.0^{a} ±2.58	429.1^{a} ±2.46	426.5^{a} ±2.52	424.6^{a} ±2.23	417.8^{b} ±2.47	
	N1/88	415.5^{a} ±3.03	427.1^{b} ±2.79	428.4^{a} ±2.32	419.3^{a} ±2.38	422.7^{a} ±2.32	419.4^{b} ±2.24	
Shell Thickness (μm)	Control	9.33^{a} ±0.06	9.55^{a} ±0.04	10.05^{a} ±0.05	9.74^{a} ±0.04	9.72^{a} ±0.04	9.70^{a} ±0.04	0.0010
	T	9.35^{a} ±0.07	9.87^{a} ±0.07	10.15^{a} ±0.07	9.76^{a} ±0.06	9.70^{a} ±0.06	9.53^{b} ±0.06	
	N1/88	9.34^{a} ±0.08	9.64^{a} ±0.07	9.95^{a} ±0.07	9.67^{b} ±0.06	9.73^{a} ±0.06	9.50^{ab} ±0.05	

Mean ± S.E. P values are for the interaction between challenge group and time.
Values in a row with different superscripts are significantly different from one another

Table E2 The effect of challenge on egg internal quality.

		Before	1 Wk	2 Wks	3 Wks	4 Wks	5 Wks	P Value
Albumen Height mm	Control	9.34[a] ±0.06	9.08[b] ±0.06	8.56[b] ±0.06	8.92[b] ±0.07	8.98[b] ±0.07	9.00[b] ±0.07	<0.0001
	T	9.25[a] ±0.08	8.97[b] ±0.09	9.21[a] ±0.09	8.79[b] ±0.09	9.03[b] ±0.09	9.13[b] ±0.09	
	N1/88	9.43[a] ±0.08	9.39[a] ±0.09	9.31[a] ±0.10	9.17[a] ±0.09	9.47[a] ±0.10	9.71[a] ±0.09	
Haugh Units	Control	95.55[a] ±0.31	94.05[b] ±0.32	91.35[b] ±0.32	92.98[b] ±0.37	93.28[b] ±0.35	93.38[b] ±0.67	<0.0001
	T	94.96[a] ±0.40	94.10[b] ±0.44	94.98[a] ±0.43	92.99[b] ±0.48	94.16[b] ±0.44	94.53[b] ±0.49	
	N1/88	95.89[a] ±0.38	95.72[a] ±0.46	95.21[a] ±0.47	94.66[a] ±0.46	96.09[a] ±0.47	97.16[a] ±0.46	
Yolk Colour	Control	11.21[a] ±0.04	11.03[a] ±0.04	10.52[a] ±0.04	10.71[a] ±0.04	11.09[a] ±0.04	11.00[a] ±0.03	<0.0001
	T	11.06[b] ±0.05	10.87[b] ±0.05	10.60[a] ±0.05	10.70[a] ±0.05	10.88[b] ±0.05	10.98[a] ±0.05	
	N1/88	11.27[a] ±0.05	11.12[a] ±0.05	10.60[a] ±0.06	10.72[a] ±0.05	10.64[c] ±0.06	10.83[b] ±0.05	

Mean ± S.E. P values are for the interaction between challenge group and time.
Values in a row with different superscripts are significantly different from one another

Table E3 Egg shell quality for the different vaccination groups

		AAA	AVA	VVV	VAV	P-value
Deformation (μm)	Control	263.20[a] 2.77	262.32[a] 2.29	269.92[a] 3.33	265.02[ab] 3.15	0.5766
	T	264.51[a] 5.34	263.19[a] 3.84	273.08[a] 4.80	257.86[b] 2.87	
	N1/88	265.75[a] 4.76	262.41[a] 4.44	277.53[a] 4.76	273.79[a] 4.65	
Breaking Strength (N)	Control	42.48[a] 0.92	41.52[a] 0.31	43.08[a] 0.32	40.90[b] 0.31	0.1671
	T	41.74[a] 0.44	42.23[a] 0.42	42.41[a] 0.50	42.18[a] 0.51	
	N1/88	41.98[a] 0.47	40.99[a] 0.51	42.24[a] 0.39	42.25[a] 0.48	
Reflectivity (%)	Control	42.37[a] 0.60	41.54[a] 0.58	42.41[a] 0.56	42.00[a] 0.62	<0.0001
	T	41.59[a] 0.89	41.78[a] 0.91	42.15[a] 0.79	42.76[a] 0.87	
	N1/88	40.57[a] 0.76	43.42[a] 0.95	40.71[a] 0.79	42.64[a] 0.87	
Egg Weight (g)	Control	63.15[a] 0.22	62.82[a] 0.21	62.64[a] 0.21	62.32[a] 0.19	<0.0001
	T	60.33[c] 0.33	62.60[ab] 0.28	61.72[b] 0.25	60.19[b] 0.24	
	N1/88	61.84[b] 0.29	61.89[b] 0.27	62.03[ab] 0.27	62.38[a] 0.25	
Shell Weight (g)	Control	6.09[a] 0.03	6.02[ab] 0.03	6.15[a] 0.03	6.00[a] 0.03	<0.0001
	T	5.86[c] 0.04	6.05[a] 0.04	5.98[b] 0.04	5.90[b] 0.05	
	N1/88	5.98[b] 0.03	5.93[b] 0.04	5.95[b] 0.03	6.02[a] 0.04	
% Shell	Control	9.66[a] 0.04	9.60[a] 0.04	9.83[a] 0.04	9.64[b] 0.04	0.0032
	T	9.72[a] 0.05	9.68[a] 0.05	9.70[b] 0.06	9.80[a] 0.07	
	N1/88	9.70[a] 0.05	9.59[a] 0.05	9.62[b] 0.05	9.65[ab] 0.06	
Shell Thickness (μm)	Control	425.2[a] 1.53	422.6[a] 1.62	430.7[a] 1.48	421.5[a] 1.60	0.0005
	T	422.3[a] 2.10	425.6[a] 1.92	425.5[b] 1.91	424.9[a] 2.38	
	N1/88	424.1[a] 1.69	419.8[a] 2.13	420.8[b] 2.07	423.3[a] 2.37	

Mean ± S.E. P values are for the interaction between challenge group and vaccination group.
Values in a row with different superscripts are significantly different from one another

Table E4 Egg internal quality for the different vaccination groups

		AAA	AVA	VVV	VAV	P-value
Albumen Height (mm)	Control	9.07^{b} 0.06	8.88^{a} 0.06	8.96^{b} 0.06	9.01^{a} 0.04	<0.0001
	T	9.08^{b} 0.07	8.85^{a} 0.07	9.19^{a} 0.07	9.15^{a} 0.08	
	N1/88	9.40^{a} 0.06	9.87^{b} 0.07	9.56^{a} 0.08	9.14^{a} 0.08	
Haugh Units	Control	93.77^{c} 0.29	92.88^{b} 0.30	93.25^{b} 0.30	93.83^{b} 0.23	<0.0001
	T	94.64^{b} 0.32	92.94^{b} 0.34	94.83^{a} 0.36	94.80^{a} 0.42	
	N1/88	95.96^{a} 0.28	98.05^{a} 0.31	94.87^{a} 0.44	94.37^{ab} 0.39	
Yolk Colour Score	Control	10.91^{a} 0.03	10.90^{a} 0.03	10.93^{a} 0.03	10.96^{a} 0.03	0.0429
	T	10.90^{a} 0.04	10.83^{a} 0.04	10.80^{b} 0.04	10.86^{a} 0.04	
	N1/88	10.83^{a} 0.05	10.95^{a} 0.05	10.79^{b} 0.05	10.88^{a} 0.05	

Mean ± S.E. P values are for the interaction between challenge group and vaccination group.
Values in a row with different superscripts are significantly different from one another

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