PRELIMINARY STUDIES ON THE EFFECTS OF INFECTIOUS BRONCHITIS ON UNVACCINATED LAYING HENS

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Summary

Preliminary studies were conducted on unvaccinated laying hens to investigate the effects of two strains of infectious bronchitis virus (T strain and N1/88 strain) on unprotected birds during lay. Clinical symptoms associated with the respiratory system were observed in the T-strain and N-strain groups but not the control and some birds in the T-strain group had enlarged kidneys. Feed intake tended to be depressed in the challenged birds and production was reduced in the T-strain group at 3 weeks post-challenge. However, there were relatively few effects of challenge on egg quality and no significant effects on excreta moisture. IBV antibody titres increased in response to challenge in the T-strain group but only some challenged birds produced antibodies in the N-strain group. Virus was re-isolated from the kidneys of T-strain birds at all sampling times post-challenge but only at 6-16 days for the N-strain group. Further studies are planned to document the effect of IBV challenge on unvaccinated birds.

I. INTRODUCTION

It is approximately 40 years since nephropathogenic strains of infectious bronchitis (IB) were isolated in Australia by Cumming (1963, 1965). Since that time, IB has been known to affect the respiratory system and oviduct as well as the kidneys of chickens (Jordan, 1996). The effect of infectious bronchitis virus on the oviduct of laying hens has been the subject of extensive conjecture and the effects of IB on egg quality that have been reported overseas (Jordan, 1996), have not been directly demonstrated in the Australian environment.

The current studies used White Leghorn birds that had been maintained in isolation from day-old and not vaccinated against IB, for the purposes of producing fertile eggs for other studies. Birds were maintained IB free until 65 weeks of age, at which time they were exposed to one of two strains of IB: T strain or N1/88 strain. T strain is a strongly nephropathogenic virus whereas N1/88 strain has a greater affinity for the respiratory system.

These naïve birds were used as a model for a commercial laying hen that has not been effectively vaccinated against infectious bronchitis virus. The effects of IBV on these birds would be expected to represent the most severe effect that could be expected in commercial birds.

II. MATERIALS AND METHODS

Day-old White Leghorn chicks were obtained from the Nulkaba Hatchery near Cessnock, NSW and transferred to isolation pens at the University of New England, Armidale, NSW. The birds were reared according to standard commercial practice. Birds remained in the isolation sheds and blood samples were taken at intervals to confirm that there were no antibodies to IB. The birds remained IB antibody negative to 65 weeks of age.

At 65 weeks of age, birds were divided into three groups: a control group in which birds were transferred to individual cages in small isolation sheds; a T-strain group with birds

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being transferred to individual cages in a large isolation shed and inoculated intraocularly
with T-strain IBV; and an N-strain group with birds being transferred to individual cages in a
separate large isolation shed and inoculated intraocularly with N1/88-strain IBV. The dose of
each challenge virus was adjusted to ensure a final estimated dose of $2 \times 10^5$ EID$_{50}$ per bird.

Within each treatment group, some birds were maintained throughout the experiment
for the purposes of measuring feed intake, egg production, egg quality and excreta moisture. A
subsample of these birds (6 per treatment group) had blood samples taken prior to
challenge and three weeks following challenge for measurement of IBV antibody titre by
IDEXX ELISA. The other birds were sacrificed at 3, 6, 10, 13, 16 and 21 days postchallenge
for assessment of histopathological changes. Kidney tissue was stored frozen for later re-
isolation of virus. For the birds that were sacrificed, blood samples were taken prior to
challenge and then at the time of sacrifice for measurement of IBV antibody titre by serum
neutralization and IDEXX ELISA.

Re-isolation of virus was attempted from frozen kidney tissue by injection of kidney
extract into the allantoic cavity of 9-day old embryos for a total of five passages. If at least 3
of the 5 embryos were dead or virus-affected, the sample of kidney was scored as positive for
re-isolation of virus.

Data presented in Figures 1-3 were analysed by ANOVA. Fisher’s protected LSD
was used to separate means when significant main effects were observed. Figure 3 presents
percentage of samples positive for the presence of IBV.

III. RESULTS

No clinical symptoms were observed in the Control group of birds. However, rales
and other symptoms of respiratory disease were observed in the N-strain group from 4 to 6
days postchallenge and in the T-strain group from 2 to 7 days postchallenge. However, the
birds recovered from these symptoms.

Feed intake varied significantly over the weeks of the experiment. In the first week
post challenge, feed intake was lower for the N and T groups, in comparison to the control
(Figure 1).

![Feed intake graph](image1)
![Hen day production graph](image2)

Figure 1: Feed intake (g/bird/day)   Figure 2: Hen day production (eggs/hen/100d)

Production declined in all treatment groups during weeks 1 and 2 (Figure 2). The
decline in production in all groups may be attributed to the very hot weather that was
experienced during those two weeks. During week 3 of the experiment, production had
improved in the control and N groups but was still significantly depressed in the T group and
this trend continued until the end of the experiment.
There were significant main effects of treatment group and week of experiment on measurements of egg shell quality but no statistically significant interactions between group and treatment group, indicating that challenge with either T-strain or N-strain infectious bronchitis virus had little effect on egg shell quality in this study. A similar pattern was found for egg internal quality as measured by albumen height and Haugh Units. There were significant main effects and a significant interaction for yolk colour, with yolk colour in the T group being generally lower in the post-challenge phase of the experiment.

Excreta moisture varied over the weeks of the experiment mainly as the result of changes in ambient temperature and humidity. However, there was no difference between treatment groups and no significant interaction between treatment group and week of experiment.

All birds tested negative to the presence of IBV antibodies, by both serum neutralization testing and ELISA, prior to the challenge and the control group birds all remained negative. For the N group, IBV antibody titres were negative by serum neutralization and ELISA until 21 days post-challenge in the birds that were sacrificed and were negative at 3 weeks post-challenge in the birds that were maintained throughout the experiment (Figure 3). For the T group birds that were sacrificed, all birds were positive by ELISA from 10 days post-challenge. For the T group birds that were maintained throughout the experiment, there was a large and highly statistically significant (P<0.0001) increase in IBV antibody titre, as measured by ELISA, at 3 weeks post-challenge (Figure 3).

![IBV antibody ELISA titres for birds maintained throughout the experiment](image1)

![Percentage of kidney extracts positive for presence of virus](image2)

Virus was not re-isolated from any kidneys from the control group of birds. The percentage of birds from which virus was re-isolated in the T group increased to 100% by 6 days post challenge and was still at 33.3% 21 days post challenge (Figure 4). At the same time, it took longer (16 days post-challenge) for all birds to have virus present in the kidneys in the N group and no birds tested positive for virus in the kidneys by 21 weeks post challenge. Enlarged kidneys were not observed in the Control and N strain group but were observed in individual birds in the T strain group at 10, 13 and 21 days post challenge.

IV. DISCUSSION

The presence of clinical symptoms in birds from the N-strain and T-strain groups, as well as the large increase in IBV antibody titre in the T-strain group indicate that a significant viral challenge was delivered to the experimental groups of birds. However, the effects of challenge on feed intake and egg production were relatively mild. Feed intake tended to be lower in the N and T groups in the first week post-challenge. Egg production was
significantly lower for the T group at three weeks post challenge and was depressed for the remainder of the experiment. Excreta moisture was not statistically significantly affected by IBV challenge.

There were relatively few effects of IBV challenge on egg internal quality and egg shell quality. The lower yolk colour of eggs from the T-strain group following challenge was probably due, at least in part, to a reduction in feed intake. However, it is possible that there were also effects on other aspects of functioning.

The serum samples taken from birds that were sacrificed were negative for IBV antibody titres throughout the experiment in the control group. For the N-strain birds that were sacrificed, all serum samples were negative for IBV antibodies as measured by serum neutralization or IBV antibody ELISA titre until 21 days post-challenge. However, for the N-strain birds that were maintained throughout the experiment, all serum samples taken prior to challenge and at 3 weeks post-challenge were negative for IBV antibody titres, as measured by ELISA. For the T-strain birds that were sacrificed, most plasma samples were positive for IBV antibody by both serum neutralization and ELISA from 10 days post-challenge until the end of the experiment. For the T-strain birds maintained throughout the experiment, serum samples were negative for IBV antibodies prior to challenge and there was an increase in IBV antibody titre at 3 weeks post-challenge. These results indicate that T-strain evokes a greater antibody response, in birds that have not previously been vaccinated, than does N-strain IBV. This is emphasized in the results from the birds that were maintained throughout the experiment. The IBV antibody titres remained negative in both the Control and N-strain groups, although there was a numerical increase in the titres of the N-strain group. It appears that a single challenge by N-strain IBV in unvaccinated birds is not necessarily sufficient to induce an antibody response. However, for the T-strain group, there was a very large increase in IBV antibody titre at 3 weeks post-challenge.

Virus was not re-isolated from kidney tissue in any of the control group of birds. For the N-strain group, virus was re-isolated from 6 days post-challenge, reaching a peak of 100% of birds at 16 days post-challenge. However, virus could not be isolated at 21 days post-challenge. In the T-strain group, virus was reisolated from the kidneys of at least some birds at all days of sampling and was found in all birds at 6-13 days post-challenge. It appears that T-strain replicates in the kidneys more quickly following challenge than does N-strain. This is probably to be expected as T-strain is nephropathogenic whereas N-strain is is thought to have a greater affinity for the respiratory system.

The enlarged kidneys observed in some birds from the T-strain group are indicative of histopathological damage in these organs. The results of histopathological examination of tissue from the birds euthanased in this study are reported in Chousalkar and Roberts (this volume). Future experiments are planned to evaluate the effect of IBV challenge in unvaccinated brown egg layers that have come into lay. The results of such an experiment will assist in the identification of the impact of an intercurrent IBV infection on egg quality in commercial laying birds.

REFERENCES