Effects of two strains of infectious bronchitis virus on unvaccinated laying hens

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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 at one week postchallenge 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 except that yolk colour was depressed in the T-strain group. There were no significant effects on excreta moisture for any group. 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 within 3 weeks post-challenge. 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. Sequential histopathological changes revealed that IBV multiplies initially in the Harderian gland, then in the tracheal mucosa and simultaneously in the kidney and oviduct. The severity and persistence of lesions were greater in the shell gland and kidney of T infected birds whereas, in the trachea and Harderian gland, the effects of the two IBV strains were similar. N1/88 seemed to be more pathogenic for the magnum region of the oviduct.

Keywords: infectious bronchitis; laying hens; histopathology; oviduct; IBV antibody titres

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 oviduct (Sevoian & Levine, 1957; Crinion et al., 1971a,b) as well as the respiratory tract and kidneys of chickens (McMartin, 1993; 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 (Broadfoot et al., 1956; 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.
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, Australia. 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 (by ELISA and serum neutralisation) 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 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 (six 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. IDEXX ELISA antibody titres of more than 396 are considered positive.

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. Harderian gland, trachea, kidney, magnum and shell gland were fixed in 10% neutral buffered formalin. The tissues were processed by standard histological procedures, embedded in paraffin, and 5 micron sections cut. All the sections were stained with haematoxylin and eosin, in addition, some of the kidney and magnum sections were stained with alcian blue. All the stained slides were viewed by light microscopy.

Data presented in Figures 1-4 were analysed by ANOVA. Fisher’s protected LSD was used to separate means when significant main effects were observed. Figure 5 presents percentage of samples positive for the presence of IBV.

Results and discussion

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 and 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).

![Figure 1: Feed intake (g/bird/day)](image1.png)  ![Figure 2: Hen day production (eggs/hen/100d)](image2.png)
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 (Figure 3).

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 neutralisation 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 neutralisation 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 4). 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<0.01) increase in IBV antibody titre, as measured by ELISA, at 3 weeks post-challenge (Figure 4).
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 5). 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 days post challenge. Enlarged kidneys were not observed in the Control and N strain groups but were observed in individual birds in the T strain group at 10, 13 and 21 days post challenge.

In control birds, the main features in the Harderian gland were some plasma cells in the subepithelium, intact collecting duct epithelium and acinar epithelium, occasional plasma cells and lymphocyte infiltration around the blood vessels in the glandular interstitium. In T strain infected birds, on 3 day p.i., there was moderate infiltration of plasma cells and globular leukocytes, the acinar epithelium was moderately damaged but the collecting duct epithelium was severely damaged. On 6, 10, and 13 days p.i., globular leukocytes and lymphoid cells around blood vessels were intense. On 16 and 21 days p.i. most of the ductal and acinar epithelium had regenerated, the number of globular leukocytes was reduced, but the lymphocyte infiltration in the interstitium was still common. Exfoliative epithelium, along with inflammatory cells, was seen occasionally in the duct lumen at 10 days p.i. and for the remainder of experiment. Migration of lymphocytes and heterophils into the subepithelium was mild on 3, 13 16 and 21 days p.i. but moderate at 6 and 10 days p.i. In N1/88 infected birds, most of the lesions were similar to those of T strain infection but the lesions were less severe.

Normal tracheal epithelium, with healthy cilia and mucus glands, were seen in the control birds (Randall and Reece, 1996). There were no microscopic changes at three days p.i. in both T and N1/88 groups except for lymphocytic infiltration and dilatation of blood vessels in the lamina propria. Severe pathology occurred mainly from day 6 in the form of severe loss of cilia, mucus glands and goblet cells, changes in the mucosal epithelium, oedema in the subepithelium and occasional heterophilic exudate in the trabecular lumen. Most of the above lesions persisted in moderate form in both infected groups. On day 13 p.i., most of the cilia and the epithelium had regenerated. The hypertrophied glands were normal with occasional heterophilic exudate in the lumen. Goblet cells were present in good number in T infected Leghorns but moderately absent from the N1/88 infected group. On days 16 and 21 most of the tracheas appeared normal. However, severe thickening of the mucosa with infiltration of lymphocytes were prominent from days 13 to 21 p.i. Moderate heterophilic exudate was also present in the lumen of the trachea in some N1/88 infected birds up to 21 days p.i.

The main kidney lesions consisted of necrosis of proximal convoluted tubules, distension of distal convoluted tubules, necrotic foci, infiltration of heterophils and lymphocytes in the interstitial space, oedema of Bowmans capsule, urate and granulocytic casts in collecting ducts and spheroids. The lesions were more apparent on the 10th day p.i. in N1/88 infected birds. The pathology continued up to day 13 in both the infected groups and, at 16 to 21 days of infection, most of the tissues had regenerated, although oedema in Bowmans capsule and necrotic foci persisted. Spheroids were common only in T strain infected birds.

All the parts of oviduct in the control birds appeared normal throughout the experiment. In the magnum and shell gland pouch of T-infected birds, the first feature to appear was lymphoid cell infiltration around the blood vessels in the muscular layer from the 10th day p.i. However, in the magnum of N1/88 infected birds, prominent changes appeared from day 6 p.i. On day 10 p.i., severe cilia loss and oedema in the sub epithelium were main findings. Glandular dilatation in shell gland pouch was severe in T as compared to N1/88 infected birds however the opposite was recorded in the magnum. Alcian blue staining in magnum of infected birds showed loss of mucopolysachrides in major areas of mucosal cells. From 13 to 21 days p.i., cellular infiltration in lamina propria and muscularis layers was at a peak. Most of the tissues had regenerated at 21 day p.i.

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 such as lipid absorption and mobilisation.

In the control group, 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 neutralisation 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 IBV. This is probably to be expected as T-strain is considered nephropathogenic whereas N-strain is regarded as having 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.

In both challenged groups (T and N1/88), the severity and time frame of lesions in the Harderian gland were almost the same which indicates that both strains are equally pathogenic for the Harderian gland. Our finding regarding regeneration of the ductal epithelium agrees with Toro et al. (1996). Histological lesions observed in the trachea are similar to those described previously (Chen et al., 1996). Lesions were similar for both IBV strains indicating similar predilection of both strains for the trachea. The histopathological changes observed in the kidney match previous findings (Fulton et al., 1993). T strain was more nephropathogenic (Chong et al., 1982) as compared to N1/88 in Leghorn birds. Most of the changes in the oviduct were noticeable on the 10th day p.i., a finding that is in accordance with Sevoian & Levine (1957). Glandular dilatation may be the contributory factor in albumen thinning (Butler, 1972). The moderate inflammatory cell debris in the lumen of the oviduct may lead to the presence of meat spots in egg albumen as reported by McDougall (1968) although pathogenesis of misshapen, soft shelled eggs and the mechanism of cessation or reduced egg production in IBV infection needs further investigation. The duration and severity of effects suggests that, in the oviduct, T strain has more affinity and pathogenicity for the shell gland where as N1/88 was more pathogenic in the magnum. After experimental challenge with IBV, the sequential observations by histopathology suggest that virus replicates first in the Harderian gland, then tracheal mucosa and then simultaneously replicates in kidney and oviduct.

T-strain IBV had more deleterious effects than N-strain with more pronounced clinical symptoms, a greater drop in production, much greater antibody response and replication of virus in the kidneys over a longer period of time. Further 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


