HISTOPATHOLOGY OF TWO SEROTYPES (T & N1/88) OF AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) IN VACCINATED AND UNVACCINATED BIRDS

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Summary

The histopathology of Harderian gland, trachea, kidney and oviduct was studied in vaccinated and unvaccinated birds exposed to T and N1/88 strain of Infectious Bronchitis Virus (IBV). The trachea and kidney of vaccinated birds were protected to a moderate extent but the oviduct to only a small extent. The 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 trachea and Harderian gland the effects of the two IBV strains were similar. The N1/88 strain seemed to be more pathogenic for magnum of vaccinated and unvaccinated birds.

I. INTRODUCTION

Infectious bronchitis is an acute, highly contagious and primarily respiratory infection in chickens. Infectious bronchitis virus (IBV) has a great economic impact on the layer industry as it affects egg production. Besides respiratory lesions, early exposure to IBV causes extensive damage to the reproductive tract (Broadfoot et al., 1956).

IBV strains vary greatly in their tissue tropism with T strain being regarded as nephropathogenic and N1/88 as respiratory. Many workers reported IBV as a respiratory syndrome (McMartin, 1993) with clinical signs being difficulty in breathing, rales, coughing, or sneezing, with or without nasal discharge. In layers, infection at an early age causes permanent damage to the oviduct (Crinion et al., 1971), along with some respiratory signs. In adult laying hens, respiratory signs may be in milder form and can remain unnoticed. The virus usually causes reproductive disorders with decline in egg production accompanied by soft shelled and misshapen eggs, inferior shell quality and thin and watery albumen.

The pathology of the respiratory tract and kidney has been studied by many workers (Chen and Itakura, 1996) while the pathology of the reproductive tract has been investigated on by only a few researchers (Sevoian and Levine, 1957; Crinion et al., 1971). The objective of this work is to describe the pathology and time frame of IBV effects in laying birds. This paper will concentrate on the Harderian gland, trachea, kidney and different parts of the oviduct.

II. MATERIALS AND METHODS

In total, 74 birds were used in this experiment with details of experimental design presented in Table 1. All the HyLine Grey birds (HL) were vaccinated with commercial vaccine at the age of day old, four weeks and twelve weeks whereas all White Leghorn (L) birds were kept unvaccinated. Leghorns at the age of 65 weeks and HyLine birds at the age of 110 weeks were challenged with two different Australian strains of virus, T and N1/88 (obtained from Dr. Jagoda Ignatovic, CSIRO, Geelong). Three unvaccinated birds (L) and

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two vaccinated birds (HL) were killed and examined on days 3, 6, 10, 13, 16, 21 post infection (p.i.). 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 μm sections cut. All the sections were stained with haematoxylin and eosin. In addition, some of the kidney and magnum sections were stained with alcin blue. All the stained slides were viewed by light microscopy.

Table 1. Experimental design.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Vaccinated</th>
<th>Control</th>
<th>Challenged: T strain</th>
<th>Challenged: N1/88 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leghorn (L)</td>
<td>44</td>
<td>No</td>
<td>7</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>HyLine Grey (H)</td>
<td>30</td>
<td>All</td>
<td>6</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

III. RESULTS

a) Harderian gland

In control Leghorn birds, the main features 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 control HyLine birds, most of the findings were similar to the control Leghorn birds except for the migratory infiltration of lymphocytes in the interstitium found in the HyLine birds.

In T strain infected Leghorn birds, on 3 day p.i., there was moderate infiltration of plasma cells and plasma cells with RB, the acinar epithelium was moderately damaged but the collecting duct epithelium was severely damaged. On 6, 10, and 13 days p.i., plasma cells with RB 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 RB bearing plasma cells 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 Leghorn birds, most of the lesions were similar to those of T strain infection but the lesions were less severe.

In both T and N1/88 HyLine Grey birds, the lesions were severe on 3 and 6 days and moderate at 10, 13 and 21 days p.i. However there was regeneration of the collecting duct epithelium on day 10 p.i. In HyLine birds, the amount of secretion in the collecting duct lumen was increased throughout the experiment as compared to control HyLine birds.

b) Trachea

Normal tracheal epithelium, with healthy cilia and mucus glands, were seen in the control Leghorn birds (Randall and Reece, 1996).

There were no microscopic changes at three days p.i. in both T and N1/88 Leghorn 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 tracheal 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 Leghorn birds up to 21 days p.i. The severity of lesions in infected HyLine birds was less than infected Leghorn birds. However, in all HyLine birds including the control group, there was extensive thickening of the mucosa with lymphoid nodules throughout the experimental period.

c) Kidney

In Leghorn birds, 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 Leghorns. In infected HyLine birds, all the above findings were mild or moderate. Necrotic foci along with lymphoid cell infiltration persisted until the end of experiment.

d) Oviduct

In magnum and shell gland pouch of T-infected HyLine and Leghorn 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 magnum of N1/88 infected Leghorns, prominent changes appeared from day 6 p.i. In both infected groups, 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 Leghorns however reverse was recorded in magnum of Leghorn birds. Alcian blue staining in magnum of infected Leghorns showed loss of mucopolysaccharides 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. All the parts of oviduct in the control Leghorns appeared normal throughout the experiment.

All the above findings were moderate but consistent in shell gland but severe in magnum of all infected HyLine birds.

IV. DISCUSSION

In both challenged groups (T and N1/88) of Leghorn birds, 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 although regeneration occurred more quickly in the HyLine birds as compared to the Leghorns. 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 and Itakura, 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 and Apostolov, 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 and Levine (1957). Glandular dilatation may be the contributory factor in albumen thinning (Butler et al., 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. The histological findings were more severe in infected Leghorns as compared to HyLine birds which indicates that the vaccine has protected the trachea to a moderate extent (Box et al., 1980), kidney to a large extent (Cavanagh, 2003) and oviduct to only a small extent.

In both the IBV infected Leghorn groups, histopathological lesions were more severe in Harderian gland and trachea than for infected HyLine birds, but were not devastating in kidney and oviduct as described in earlier literature. This may be due to an intrinsic factor like age influencing the pathogenesis for kidney (Albassam et al., 1986) and oviduct (Crinion and Hoestad, 1972). 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.

REFERENCES

McDougall, J. S. (1968). The Veterinary Record, 83: 84-86.