PERSISTANCE AND MORPHOPATHOLOGICAL STUDIES ON INFECTIOUS BRONCHITIS IN CHICKENS IN PAKISTAN
(With 4 Tables & 7 Figs.)

By

T. JAVED; M. SIDDIQUE* and A. HAMEED

(Received at 28/10/1990)

SUMMARY

During one year period 432 chicken flocks with variable disease conditions were surveyed for the prevalence of various respiratory problems. Among 432 flock surveyed 18 (4.16%) were positive for IB. Regarding age-wise incidence maximum (35.71%) infection was recorded in 1-5 week age group. Season wise maximum (66.66%) incidence was recorded in winter season. Maximum IBV was isolated from lungs, trachea and kidneys. IBV affected embryos showed curling and dwarfing along with severe haemorrhages on the egg shell membrane. Different physicochemical parameters of eggs from hens infected with IB were significantly different from normal eggs. Studies of gross and histopathological alterations in different visceral organs of chickens affected with infectious bronchitis were studied. In IB trachea, larynx, lungs, oviduct and kidneys were the target organs.

* Department of Microbiology, University of Agricultural Faisalabad

INTRODUCTION

Viral diseases of respiratory tract, like infectious bronchitis, newcastle disease and infectious laryngotracheitis have greater economic significance (TARIQ, et al. 1989). Clinical manifestations and gross pathological changes in these diseases are very similar, thereby there is difficulty in differential diagnosis. Direct air born transmission is the most usual method of spread. Infectious bronchitis is egg borne and infection is transmitted to the next generation causing serious implications in hatcheries (LUKERT, 1980).


Infectious bronchitis is highly contagious acute infection of upper respiratory tract affecting birds mostly up to 3 months of age (ROSZKOWSKI, et al. 1986). Infection may however, persist in some birds for many months and outbreaks may occur under any stress conditions (GAFFAR ELAMIN, 1986). There may be aberrant development of the oviduct with partial or complete failure of the development of oviduct resulting in reduction in egg production and poor quality eggs (JAVED and SIDDIQUE, 1990).

Keeping in view the importance of this disease of the respiratory tract, this project has been designed to study the incidence, clinical findings, gross and histopathological details of infectious bronchitis.

MATERIAL and METHODS

Survey:

Various poultry farms in and around Faisalabad, Pakistan were surveyed and the birds showing clinical respiratory distress were selected to study the incidence of IB by isolation, serology, and pathology of Infectious Bronchitis (IB). A total of 432 flocks were taken for survey. These flocks consist of 135 broiler flocks, 7 broiler breeder flocks and 290 layer flocks. Details about the history of flock and managemental conditions were recorded. Serum samples, eggs, respiratory exudates and tissues from sick and dead birds were collected for further studies.
INFECTIONOUS BRONCHITIS IN PAKISTAN

Serology:
Among serological studies lyophilized infectious bronchitis antigen prepared from strain H-52 was obtained with the courtesy of centre of Research and Bio-preparation, volunter, pasteur Institute, Bucharest, Romania. Antigen was propagated in embryonated chicken eggs of 9-11 days. Haemagglutination with chicken R.B.Cs. Trypsin induced infectious bronchitis haemagglutional test was performed (KAHRAMAN, 1988). Indirect haemagglutination Inhibition (IHI) test, Agar gel ppt test (WITTER, 1962), Micro ppt test (BAUDITZ, 1963) were main confirmatory tools by using IB positive antisera.

Virus Isolation:
Respiratory exudates and triturated tissue samples were inoculated for isolation of virus and study the embryonic changes.

Gross and Histopathological Studies:
Different visceral organs from birds confirmed through different tests as IB affecteed were examined for the gross and histopathological studies. Selected pieces of 5-6 mm size from various morbid organs were fixed in 10 per cent formation solution. The fixed tissue pieces were dehydrated through ascending grades of ethyl alcohol, cleared in xylol, infiltrated in melted paraffin for 12 hours and tissue blocks were prepared. Section of 5-7 um thickness were cut with rotary microtome with disposable microtome knife (SABRI, et al. 1986).

Physico-Chemical parameters:
Physio-chemical parameters affected and that of normal hens were included, weights of egg, shell, albumin and yolk according to ZUBAIR (1985). Specific gravity of the eggs was recorded with the help of gravimeter, surface tension of the albumin with "Due Novy", torsion balance. Viscosity of albumin was done with the viscometer (FAZAL and ALI, 1984). Total proteins and cholesterol contents of eggs from hens suffering from IB and normal eggs were determined.

RESULTS
Are presented in tables 1-4 and figures 1-7.

DISCUSSION
During the one year period a total of 432 chicken flocks with variable disease conditions were surveyed for the prevalence of IB, out of them 135 (31.25%) broiler flocks and 7(1.62%) broiler breeder flocks. Antibodies against infectious bronchitis

were determined by indirect haemagglutination inhibition, microprecipitation and agar gel precipitation tests. Out of 432 flocks surveyed, 18 (4.16%) flocks were positive for antibodies against IB. Among 290 layer flocks 10 (3.44%) were sero positive for IB. Similarly in broiler flocks incidence of IB was 5.18 percent. Out of 7 breeder flocks surveyed, only one was positive for infectious bronchitis. GAFFAR ELAMIN, et al. (1986) reported 33 per cent prevalence of infectious bronchitis in Sudan. Incidence of IB in different types of birds is summarised in Table I.

Regarding age wise incidence of infectious bronchitis, 11 (22.00%) flocks were of 1-5 weeks age; 5 (35.71%) of layers and 6 (17.14%) of broilers. Among the age group of 6-10 weeks, only 1 (6.25%) broiler flock was positive (Table II). Only one (3.3%) layer flock was positive in between the age of 11-20 weeks and 3 (12.50%) between 21-40 weeks. In age group 41-60 weeks one of layer and chicken breeder flocks (each) was positive. Among the area wise incidence of infectious bronchitis, it varied from place to place. Prevalence of particular viral diseases in particular areas has documented by MALIK and VERMA (1969).

Incidence of infectious bronchitis influenced by the change in seasons. Maximum incidence was recorded in winter season (66.66%), followed by 17.24 per cent in spring season, while it was lower in summer (3.44%) and autumn (11.11%). In layers it was maximum (20.0%) in broiler in winter season (JAVED and SIDDIQUE, 1987).

Isolation from 18 positive flocks was confirmed by different serological tests. Lungs, tracheas and kidneys were collected and preserved in glycerine. Two hundred and seventy three samples of lungs, trachea and kidneys were taken for isolation of infectious bronchitis virus. Out of total samples inoculated, 17 were positive for IB giving an isolation incidence of 6.22 per cent (Table III). Out of these samples, 10 lungs (10.90%) 5 tracheas (5.0%) and 2 kidneys (2.19%) were positive. Maximum (6.97%) isolation was from layers followed by broiler (5.95 per cent).

 Infective materials confirmed by isolation were injected into 10-12 days old embryonated hen eggs and subsequently incubated. Curling and dwarfing of the embryos was very typical in most of the cases after the 1st passage. (Fig. 1). Severe haemorrhages on the shell membrane were very common in many cases (Fig. 2). Lungs showed pneumonia changes, liver revealed typical necrotic foci (JAMIL, et al. 1986).

 Infectious bronchitis in most of the flocks started as respiratory distress, difficult breathing with stretched neck, rattling sounds, sneezing, occular and nasal discharge and these symptoms were rapidly followed by dullness and in ultimate stages development of diarrhea and high quantity of uric acid along with drop in production with mis-shapen and thin shelled eggs (Fig. 3 and 4). Morbidity was in more than 25 per cent of the flocks up to 100% while mortality varied from 3-8 per cent. Daily mortality in most of the flocks varied from 02 to 5.91 per cent. (GAFFAR ELAMIN, 1986)
INFECTIONOUS BRONCHITIS IN PAKISTAN

reported 25 per cent mortality in chicken. Recovery in most of the flocks took 2 weeks. Drop in egg production was recorded (12.84 to 37.28 per cent). Egg production came to pre infection level after 3 weeks (CAVANAGH, 1988).

Different physico-chemical parameters of eggs from hens infected with infectious bronchitis were studied. Affected flocks laid 0.5 to 1 per cent abnormal mis shaped and disfigured eggs. Approximately 50 per cent mis-shapen eggs were thin shelled and cracked easily (Fig. 5). Physico-chemical parameters including total weight, weight of shell, weight of albumin, weight of yolk, specific gravity, viscosity, surface tension, total proteins and cholesterol were compared with those of eggs from normal hens (Table V). All the physical and chemical parameters were lower in IB affected eggs as compared to normal eggs and difference was statistically highly significant. Albumin of infectious bronchitis affected eggs have spreading nature (Fig. 6) and poor storage quality (CAVANAGH, 1988).

In IB affected birds, trachea and bronchi were severely inflamed with excessive mucous in sinuses and in tracheal lumen. The lungs appeared congested and pneumatic. Some of the birds showed cloudiness of the thoracic air sacs and sinusitis. Urate crystals were also seen in some ureters. Bursa of fabricuis showed haemorrhagic spots in a few cases. These findings are in line with the findings of CHANDRA, et al. (1981).

Glandular depletion of the internal surface of the oviduct especially that of isthmus was recorded in laying birds. This may be the most important factor of mis-shapen, disfigured and irregular eggs as also reported by HAGEN and BRUNER (1961) and JAVED and SIDIQUIE (1990).

In IB affected larynx and trachea cellular infiltration with plasma cells, histiocytes and lymphocytes were the main histological lesions recorded. Vascular congestion, oedema and hyperplasia of the epithelium were also common (Fig. 7). These findings are almost in line with that of GOUFFAX, et al. (1977) and DUCATELLE, et al. (1984).

Infectious bronchitis positive lungs showed pneumatic changes, leukocytic infiltration and slight exudation. Bronchitis was common lesion observed. These findings are identical with those reported by DUCATELLE, et al. (1984).

In IB affected birds kidneys showed inflammatory changes, tubules appeared distended with crystals of uric acid. These observations are close to the findings of CHANDRA, et al. (1981) and SIDIQUIE, et al. (1987).

The picture might be aggravated by the presence of other disease. Precise study might be done with SPF.
REFERENCES


INFECTIONOUS BRONCHITIS IN PAKISTAN


### Table (1)
Incidence of infectious bronchitis among flocks with respiratory problems in different type of chickens

<table>
<thead>
<tr>
<th>TYPE OF FLOCK</th>
<th>NUMBER OF FLOCKS</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total surveyed</td>
<td>With resp. problem</td>
</tr>
<tr>
<td>Layer</td>
<td>290</td>
<td>85</td>
</tr>
<tr>
<td>Broiler</td>
<td>135</td>
<td>51</td>
</tr>
<tr>
<td>B.Breeder</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>432</td>
<td>140</td>
</tr>
</tbody>
</table>

### Table (2)
Number (Percentage) of flocks positive for infectious bronchitis in different age groups

<table>
<thead>
<tr>
<th>TYPE OF FLOCKS</th>
<th>AGE IN WEEKS</th>
<th>TOTAL (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-5</td>
<td>6-10</td>
</tr>
<tr>
<td>Layer</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>(35.71)</td>
<td>(3.57)</td>
<td>(12.50)</td>
</tr>
<tr>
<td>Broiler</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>(17.14)</td>
<td>(6.25)</td>
<td></td>
</tr>
<tr>
<td>B.Breeder</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(50.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>(22.00)</td>
<td>(3.12)</td>
<td>(3.57)</td>
</tr>
</tbody>
</table>

### Table (3)

Organic isolation of infectious bronchitis virus from different tissues of layers, broilers and B. breeder

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Breeder</th>
<th>Broiler</th>
<th>Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of Sample</td>
<td>Lungs</td>
<td>Trachea</td>
<td>Kidney</td>
</tr>
<tr>
<td>Lungs</td>
<td>20</td>
<td>29</td>
<td>43</td>
</tr>
<tr>
<td>Trachea</td>
<td>20</td>
<td>28</td>
<td>43</td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>273</td>
<td>87</td>
<td>53</td>
</tr>
<tr>
<td>Percentage of Positive Samples</td>
<td>17</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>Rate of Positive Samples</td>
<td>0.07</td>
<td>0.29</td>
<td>0.17</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>W</th>
<th>H</th>
<th>B</th>
<th>L</th>
<th>Weight of Eggshell</th>
<th>Total Weight</th>
<th>Wet Weight of Yolk</th>
<th>Gravity of Yolk</th>
<th>Wet Weight of Albumen</th>
<th>Gravity of Albumen</th>
<th>Weight of Yolk</th>
<th>Weight of Albumen</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>7.2</td>
<td>6.9</td>
<td>5.0</td>
<td>4.25 ± 0.65</td>
<td>6.06 ± 0.36</td>
<td>0.33 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>10.2</td>
<td>7.4</td>
<td>6.8</td>
<td>4.0</td>
<td>4.25 ± 0.65</td>
<td>6.06 ± 0.36</td>
<td>0.33 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>10.3</td>
<td>7.5</td>
<td>6.7</td>
<td>3.0</td>
<td>4.25 ± 0.65</td>
<td>6.06 ± 0.36</td>
<td>0.33 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>10.4</td>
<td>7.6</td>
<td>6.6</td>
<td>2.0</td>
<td>4.25 ± 0.65</td>
<td>6.06 ± 0.36</td>
<td>0.33 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>1.07 ± 0.05</td>
</tr>
</tbody>
</table>

Different Physico-chemical parameters of normal and infertile eggs.
Fig. (1): Normal (N) and IBV (D) inoculated embryo showing dwarfism and stunted growth.

Fig. (2): Haemorrhages on shell membrane in IBV inoculated embryonated egg.
Fig. (3): Mis-Shaped eggs from IB affected layer.
Fig. (4): Thin shelled egg from IB affected layer.
Fig. (5): Cracks, ridges and grooves present on the egg shell from IB affected hen.
Fig. (6): Watery albumin in IB affected egg.

Fig. (7): Trachea showing leukocytic infiltration (L) sloughing of mucosa (S) and haemorrhages (H).