Pathological study on the upper respiratory tract infection of chickens and isolation, identification of causal bacteria

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Abstract

The proportional occurrence of bacteria and pathological lesions in the nasal sinuses and trachea of dead chickens were determined during 2008-2009. Nasal sinus and tracheal swabs from 50 dead birds were collected in sterile nutrient broth. The histopathological samples were collected in 10% neutral buffered formalin and studied with light microscope. The isolation and identification of bacteria were performed by culture, staining and biochemical tests. The proportional occurrence of bacteria in trachea (n = 50) and nasal sinuses (n = 50) of dead chickens was Klebsiella sp. (6.0%), Escherichia coli (38.8%), Pasteurella sp. (8.6%), Bacillus sp. (5.2%) and Staphylococcus sp. (41.4%). Congested trachea (n=3) and mucus-filled sinuses (n = 3) of dead chickens were studied for histopathology. Microscopically, rhinitis was characterized by infiltration of macrophages, lymphocytes and few neutrophils. The epithelium of nasal passage revealed pyknotic nucleus with disruption of epithelium. There was sinusitis with purulent and necrotic changes around the nasal sinuses. The nasal sinuses were infiltrated with macrophages, lymphocytes and few plasma cells. The mucosal layer of the nasal turbinates showed pus and necrosis. There was disruption of different mucous glands with accumulation of macrophages, lymphocytes and plasma cells in the submucosa. (Bangl. vet. 2011. Vol. 28, No. 2, 60 – 69)

Introduction

In birds, several bacteria, Pasteurella multocida, Pasteurella gallinarum, Mannheimia haemolytica and Pasteurella anatipestifer, Bordetella avium and Haemophilus paragallinarum are involved in respiratory disease (Hafez, 2002). Escherichia coli are also associated with respiratory infection in chickens (Sukhon et al., 2002). Ornithobacterium rhinotracheale has recently been identified as causing respiratory tract infections in poultry and other birds (Vandamme et al., 1994; Chin et al., 2003). Concurrent infection of young poultry with Klebsiella pneumoniae increased the severity of respiratory disease (Saif, 2003). Weakness, gasping, pump-handled respiration, dyspnoea, mucous discharge and mortality, swelling of sinuses, facial oedema, tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis, drop in egg production and poor egg quality characterize the respiratory infection (Zorman et al., 2000; Canal et al., 2005).

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Confirmatory diagnosis of bacterial diseases of upper respiratory tract infection in poultry requires more data. The investigation was designed to (a) isolate and identify the bacteria from nasal sinuses and trachea and to (b) determine the pathological lesions caused by these bacteria.

Materials and Methods

Samples

A total of 100 swabs were collected for bacteriological isolation in nutrient broth from July 2008 to June 2009, of which 25 tracheal and 25 nasal sinus swabs came from KFL, Trishal, Mymensingh, 15 tracheal and 15 nasal sinus swabs from SK Veterinary Diagnostic Centre (SKVDC), Durgabari Road, Mymensingh, and 10 tracheal and 10 nasal sinus swabs from necropsy cases. Gross lesions such as congested trachea and mucus-filled sinuses of one dead bird from KFL, Trishal, Mymensingh, one from SKVDC, Durgabari Road, Mymensingh, and one from the Department of Pathology were collected for histopathology in 10% buffered formalin.

Primary culture of organisms

From the nutrient broth 50 tracheal and 50 nasal sinus swabs were placed in nutrient agar plate and incubated overnight at 37°C.

Subculture

All swab samples (50 tracheal and 50 nasal sinuses) were subcultured in nutrient agar, MacConkey agar and Eosin Methylene Blue (EMB) agar. Blood agar was used only for Pasteurella and Bacillus spp. A small amount of inoculum from the nutrient agar was spread into culture media and incubated at 37°C overnight. The organisms were identified by colony morphology, staining character and biochemical tests (Merchant and Packer, 1967; Curtis, 1985; Cheesbrough, 1985; Buxton and Fraser, 1977; Ali et al., 1998; Granum, 2001; Brooks et al., 2002; Naowarat, 2007).

Histopathology

Three tracheal and three nasal sinus tissues from three dead birds were selected for histopathology. The formalin-fixed tissues were processed following standard procedures (Luna, 1968).

Photomicrography

Photomicrographs were taken using Olympus PM-C 35 camera fitted with Olympus microscope (Olympus, Japan).

Results and Discussion

The clinical signs observed were depression, conjunctivitis, frothy oculo-nasal discharge, conjunctivitis, facial oedema, and respiratory rales. Gross pathology results are illustrated in Figs. 1-6.
Figs. 1 and 2. Frothy oculo-nasal discharge in chicken infected with *E. coli*

Figs. 3 and 4. Mucoid exudate in the nasal sinus in chickens infected with *E. coli*

Figs. 5 and 6. Congested trachea in *E. coli* infected chickens
From subculture of 100 swabs from nasal sinus and trachea, 116 isolates were found. The isolates were *Klebsiella sp.* (n = 7), *E. coli* (n = 45), *Pasteurella sp.* (n = 10), *Bacillus sp.* (n = 6) and *Staphylococcus sp.* (n = 48). Three isolates of each group were subjected to confirmation by culture, staining characters and by biochemical tests (Figs. 7-16).

**Fig. 7.** Culture of *Staphylococcus* on nutrient agar showing yellow colored colonies

**Fig. 8.** *Klebsiella sp.* shows gram negative, coccobacillary shaped organism. (Modified Gram’s staining, ×830)

**Fig. 9.** *E. coli* in Gram’s staining showing gram negative, short rod shaped organisms, arranged in single and paired (Gram’s stain, ×830)

**Fig. 10.** *Pasteurella sp.* showing gram negative and coccobacillary shaped organism with bipolar appearance (Leishman’s stain, ×830)

The bacteria found in trachea (50 swabs) with the proportion of each as a percentage of all bacteria found, in parentheses, were *Klebsiella sp.* (6.6%), *Escherichia coli* (37.7%), *Pasteurella sp.* (11.5%), *Bacillus sp.* (3.3%), *Staphylococcus sp.* (41.0%) (Table 1). In nasal sinuses (50 swabs) the proportion was *Klebsiella sp.* (5.5%), *Escherichia coli* (40%), *Pasteurella sp.* (5.5%), *Bacillus sp.* (7.3%), *Staphylococcus sp.* (41.8%); (Table 2).
Table 1. Bacteria isolated from trachea of dead chickens (n = 50)

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Organisms</th>
<th>Number of isolated bacteria</th>
<th>% of isolated bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Klebsiella sp.</td>
<td>4</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td>23</td>
<td>37.7</td>
</tr>
<tr>
<td>3</td>
<td>Pasteurella sp.</td>
<td>7</td>
<td>11.5</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus sp.</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus sp.</td>
<td>25</td>
<td>41.0</td>
</tr>
</tbody>
</table>

Fig. 11. *Bacillus sp.* showing gram positive, cylindrical rod arranged in single and long chain. (Modified Gram’s staining, ×830)

Fig. 12. *Staphylococcus sp.* showing gram positive, cocci and arranged in grape like clusters (Modified Gram’s staining, ×830)

Fig. 13. Catalase positive (gas bubble) for *E. coli*

Fig. 14. Catalase positive (gas bubble) for *Pasteurella sp.*
Table 2. Bacteria isolated from nasal sinuses of dead chickens (n = 50)

<table>
<thead>
<tr>
<th>Serial No</th>
<th>Organisms</th>
<th>Number of isolated bacteria</th>
<th>% of isolated bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Klebsiella sp.</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td>22</td>
<td>40.0</td>
</tr>
<tr>
<td>3</td>
<td>Pasteurella sp.</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus sp.</td>
<td>4</td>
<td>7.3</td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus sp.</td>
<td>23</td>
<td>41.8</td>
</tr>
</tbody>
</table>

The proportion of bacteria in trachea and sinuses together was 6% Klebsiella sp., 38.8% Escherichia coli, 8.6% Pasteurella sp., 5.2% Bacillus sp., and 41.4% Staphylococcus sp. (Table 3, Figs. 7-16).

The overall proportion of Klebsiella sp. (6%) was lower than from other authors (Ibrahim et al., 2004; Trkyilmaz, 2005). This might be due to age and breeds of the chickens, geographic variation and management, vaccination and nutrition.

The proportion of E. coli in tracheal and nasal sinus swabs (38.8%) was lower than those reported by some (Georgiades et al., 2001; Murakami et al., 2002; Yousseff et al., 2008) but higher than the findings of others (Ibrahim et al., 2004; Trkyilmaz, 2005). This might be due to age and breeds of the chickens and management. The proportion of Pasteurella sp. from trachea and sinuses was 8.6%, lower than other reports (Murakami et al., 2002; Trkyilmaz, 2005). The proportion of Bacillus sp. in trachea and nasal sinuses, 5.2%, was lower than Trkyilmaz (2005). Staphylococcus sp. formed 41.4% of the bacteria found in the trachea and nasal sinuses of dead chickens, lower than found by Trkyilmaz (2005).
The bacteria isolated from the nasal sinuses were *E. coli*, *Pasteurella sp.*, *Klebsiella sp.*, *Bacillus sp.* and *Staphylococcus*. *E. coli* are thought to cause disease in poultry including respiratory infection such as swollen head syndrome and respiratory colibacillosis; *Pasteurella sp.* is the cause of fowl cholera and induces pneumonia, chronic rhinitis, facial oedema; *Klebsiella sp.*, *E. coli* and *Pasteurella sp.* together cause pneumonia and tracheitis (Drago and Don, 1996; Fouad and Mohamed, 2008; Moursi and Sabah, 2008). According to the lesions of nasal sinuses, the disease in this study may be sinusitis.

In field cases of respiratory tract diseases in chickens, it is important to recognize that mixed infection with other organisms aggravate the clinical disease. These other micro-organisms include *Mycoplasma sp.* *H. paragallinarum* and many viruses. In this present study *Mycoplasma sp.* and *H. paragallinarum* infection were not studied.

**Histopathological study**

Gross lesions such as congested trachea and mucus-filled sinuses were studied for histopathology (Table 3). Microscopically, the section of the nose showed rhinitis characterized by excessive infiltration of macrophages, lymphocytes and a few neutrophils. The epithelium of nasal passage revealed pyknotic nucleus with disruption of epithelium. There was sinusitis with purulent and necrotic changes around the sinus with the infiltration of macrophages, lymphocytes and plasma cells. There was disruption of different mucous gland with accumulation of macrophages, lymphocytes and plasma cells in the submucosa (Figs. 17 and 18). The mucosal layer of the nasal turbinates showed pus and necrosis. There was no lesion in the trachea. Similar lesions were reported by many investigators (Nakamura *et al.*, 1997; Rocio *et al.*, 1998; Murakami *et al.*, 2002; Jaswinder *et al.*, 2005).

Fig. 17. Section of nose showing rhinitis with disruption of epithelial layer, presence of pyknotic nuclei and infiltration of macrophages, lymphocytes and few plasma cells (H&E stain, ×83)

Fig. 18. Section of nose showing focal purulent and necrotic changes of the mucosal membrane in the nasal turbinate (H&E stain, ×333)
Table 3. Bacteria identified from trachea and nasal sinuses of dead chickens

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Isolated organisms</th>
<th>Number of isolated bacteria</th>
<th>% of isolated bacteria</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Klebsiella sp.</td>
<td>116</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
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<td>38.8</td>
</tr>
<tr>
<td>3</td>
<td>Pasteurella sp.</td>
<td></td>
<td>8.6</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus sp.</td>
<td></td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus sp.</td>
<td></td>
<td>41.4</td>
</tr>
</tbody>
</table>

Conclusions

In this study, Klebsiella sp. (6.0%), Escherichia coli (38.8%), Pasteurella sp. (8.6%), Bacillus sp. (5.2%) and Staphylococcus sp. (41.4%) were isolated from trachea and nasal sinuses of chickens. Clinical signs of these affected birds were depression, conjunctivitis, frothy oculo-nasal discharge, conjunctivitis, facial oedema, and respiratory rales. Gross lesions of upper respiratory tracts were catarrhal tracheitis and rhinitis. Microscopic lesions were tracheitis, sinusitis and rhinitis characterized by infiltration of neutrophils, lymphocytes, plasma cells and macrophages at different layers of these tissues.

References


Moursi MK, Sabah KH 2008: Studies on chickens ornithobacterium sp. infection at Ismailia province. *Association for Veterinary Medical Journal* 54 357-372.


