



Bacteriological and Pathological Investigation of Nasal Passage Infections of Chickens (*Gallus gallus*)

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Abstract

An experiment was conducted at the Bangladesh Agricultural University, Mymensingh during July 2011 to May 2012 to investigate the prevalence of bacteria as well as pathological lesions in the nasal passages of dead chickens. Thirty nasal passage swabs from 30 dead birds (20 from SK Veterinary Diagnostic Centre (SKVDC) and 10 from the Department of Pathology, BAU) were collected in sterile nutrient broth. The histopathological samples (n=6) were collected in 10% neutral buffered formalin. The isolation and identification of bacteria were performed by culturing in different media, staining and biochemical tests. The collected tissues were fixed, processed, sectioned, stained and studied with light microscope. The prevalence of bacteria 30.43% *Escherichia coli*, 47.83% *Staphylococcus* sp., 13.04% *Pasteurella* sp., and 8.69% *Klebsiella* sp. were found in nasal passages (n=30) of dead chickens. Two cases of mixed infection with *E. coli* and *Pasteurella* isolates, and one case with *Klebsiella* and *E. coli* isolates were identified. Six tissue samples of grossly identifiable lesions such as congested and mucus filled nasal passages from 6 dead chickens were processed for histopathology. Microscopically, the section of nasal passages in general showed congested mucosa with excessive infiltration of heterophils and lymphocytes. The lining epithelia of nasal passage revealed acanthosis, hyperkeratosis and disruption of nasal epithelia. There was also hyperplasia of different mucous glands of nasal passages. *Klebsiella* sp. affected nasal tissues showed comparatively severe lesions than that of other bacterial infection in chickens.

Keywords: Bacteria, pathology, rhinitis, chickens

1. Introduction

Diseases of the respiratory tract have an important role in poultry. Among the systemic diseases, respiratory system diseases generally ranked first (Glisson, 1998). A mixed infection is well known as a causative agent of chronic respiratory diseases in chickens (Nunoya *et al.*, 1999). These diseases may emerge as the reflection of either primary or a multisystem diseases. In the etiology of respiratory system diseases, bacteria, virus, parasite, fungi, nutrition

and environmental factors are effective (Glisson, 1998).

In avian host, several microorganisms of the genus *Pasteurella* (*P. multocida*, *P. gallinarum*, *P. haemolytica* and *P. anatipestifer*), *Bordetella* (*B. avium*) and *Avibacterium* (*A. paragallinarum*) are involved in respiratory disease complex (Hafez, 2002). *E. coli* associated with respiratory infection in chickens have also been reported (Sukhon *et al.*, 2002). *Ornithobacterium rhinotracheale* has recently

been identified as a pathogen causing respiratory tract infections in poultry and other birds (Chin et al., 2003). Concurrent infection of young poultry with *Klebsiella pneumonia* increased the severity of respiratory diseases (Saif et al., 2003). Weakness, gasping, pump handled respiration, dyspnoea, mucous discharge and increased mortality, swelling of sinuses, facial oedema, tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis, drop in egg production, poor egg quality characterize the infection.

Recent investigation (Popy et al., 2011) in Bangladesh showed that the prevalence of bacteria 6.03% *Klebsiella* sp., 38.79% *Escherichia coli*., 8.26% *Pasteurella* sp., 5.17% *Bacillus* sp., and 41.37% *Staphylococcus* sp., were present in both trachea (n=50) and nasal sinuses (n=50) of dead chickens. Thus, it is revealed that the bacteria of nasal passages of chickens cause a great loss to the farmers. So, the present investigation has been undertaken to enrich the knowledge of stockholders with a view (a) to isolate, identify and detect the prevalence of the bacteria from nasal passage of chickens and (b) to determine the pathological lesions in nasal passage. The identified bacteria from nasal passage of chickens and pathological lesions will enrich the knowledge of veterinarian for the diagnosis of rhinitis and their treatment.

2. Materials and Methods

2.1. Samples

A total of 30 nasal swabs from layer birds of average age 6 months, of which 20 swabs from SKVDC, Durgabari Road, Mymensingh, and 10 swabs from Department of Pathology, BAU, were collected for the isolation of bacteria. Six tissue samples grossly, identifiable lesions such as congested and inflamed nasal passages of four from SKVDC, and two from Department of Pathology, BAU, were collected in 10% buffered formalin for histopathology. The study was conducted during July 2011 to May 2012.

2.2. Bacteriological media and reagents

Nutrient agar (NA), Blood agar (BA), MacConkey agar (McA) (Techno Pharm., India), Eosin methylene blue agar (Oxoid, England), Nutrient broth (HiMedia Lab. Pvt., India) were used in this study.

2.3. Different sugars

Dextrose (LOBA, India), Sucrose (Wako, Japan), Lactose (Merc, England), Maltose (Techno Phann., India), Dulcitol (Difco, USA), Xylose (Riedel-ae Hain, USA), Mannitol (Beximco. PHaema, Germany) and Inositol (LOBA, India) were used in this study.

2.4. Primary isolation of organisms

From the nutrient broth, 30 swab samples were placed in nutrient agar plate and incubated for overnight at 37°C for the growth of the organisms following routine procedures.

2.5. Subculture in selective culture media

All the 30 samples were sub-cultured in NA, McA and EMB agar. Blood agar media was used only for *Pasteurella* sp. and *Staphylococcus* sp. A small amount of inoculums from the nutrient agar was spread into different culture media and incubated at 37°C for overnight. The identification of the organisms was carried out by the different colony morphology, staining characteristics and biochemical tests as described by Naowarat (2007), Deena and Adriana (2005) and Brooks et al. (2002). On the basis of colony morphology, staining and biochemical characteristics, the organisms were isolated and identified. From the colony morphology the bacteria were categorized into different groups. From each group, representative samples were tested for biochemical test for the confirmation of the isolates.

2.6. Staining

Morphological characterization of the bacterial isolates by Gram's staining and Leishman's staining methods were used in this study (Brooks et al., 2002).

2. 7. Carbohydrate fermentation test procedure

Five basic sugars such as dextrose, sucrose, lactose, maltose and mannitol were used to determine the fermentation properties of the organisms. Three samples of tentatively diagnosed *E. coli*, 3 samples of tentatively diagnosed *Pasteurella* sp. and 2 samples of tentatively diagnosed *Klebsiella* sp. were used for carbohydrate fermentation test. The test was performed by inoculating 5 ml of nutrient broth culture of the organisms into the test tubes containing different sugar media and incubated for 72 hours at 37°C. Acid production was indicated by the color change from red to yellow of the medium and the gas production was noted by the appearance of the gas bubbles in the inverted Durham's tube (Mishra *et al.*, 2002; Prasad *et al.*, 1997; Chowdhury *et al.*, 1985).

2. 8. Voges-Proskauer, MR - VP, Indole and Catalase tests

To confirm the bacteria, above tests were performed as described by Mishra *et al.* (2002).

2. 9. Gross Pathology

The postmortem examination was carried out carefully on dead chickens. Gross tissue changes such as congested nasal passages filled with mucus were observed and recorded carefully and representative samples containing lesions were fixed in 10% neutral buffered formalin for histopathological examination.

2.10. Histopathology

Six nasal passage tissues were selected as representative samples from six dead birds for histopathological study. The formalin fixed tissues were trimmed, processed, sectioned and stained following standard procedure (Luna, 1968).

2. 11. Photomicrography

Photomicrography was taken at the Department of Pathology using photomicrographic camera (Olympus PM-C 35 Model) onto fitted with Olympus microscope (Olympus, Japan).

3. Results and Discussion

By subculturing of 30 swabs from nasal passages, 23 isolates of bacteria were identified. On the basis of colony characters, staining characters, sugar fermentation and biochemical properties, the isolates were categorized into 4 types of bacteria (Table 1). They were *E. coli* (7), and *Staphylococcus* sp. (11), *Pasteurella* sp. (3), *Klebsiella* sp. (2). Two mixed infection of *E. coli* and *Pasteurella* isolates from two samples, and one mixed infection of *Klebsiella* and *E. coli* from one sample were identified.

3.1. Colony characters, staining characters, biochemical tests and enzymatic activity test for *E. coli*.

Thirty swab samples from nasal passages were subcultured on EMB agar and MacConkey agar for isolation of *E. coli*. Among them 7 were positive for *E. coli*. In positive cases, all 7 *E. coli* produced smooth circular colonies with dark centers and metallic sheen on EMB agar (Fig. 1). The prevalence of *Escherichia coli* in nasal passage swabs of dead chickens was 30.43% (7 *E. coli* in 23 isolates from 30 nasal passage swabs), which is lower than those reported by Murthy *et al.* (2008) and higher than those of Trkylmaz (2005) and Ibrahim *et al.* (2004). In this study, prevalence of *E. coli* was less which might be due to age and breeds of the chickens and in some cases, prevalence of *E. coli* were higher which might be due to poor management. The colony characters of all isolated *E. coli* were the production of metallic sheen on EMB agar media and rose pink colored colony on the MacConkey agar media which corresponded with the findings of Sharada *et al.* (1999). *E. coli* was gram negative short rod, varying from coccoid shape to long filamentous (Fig. 2). All isolated *E. coli* in this investigation were MR and indole positive, and VP negative. Similar results were reported by Mishra *et al.* (2002).

Table 1. Identification of bacteria isolated from nasal passages of chickens

No. of isolates	Colony character	Staining character	Biochemical tests									Identified bacteria	No. of isolated bacteria	Total no. of isolated bacteria	% of isolated bacteria
			Catalase	MR	VP	Indole	Sugar media								
							Dextrose	Sucrose	Lactose	Maltose	Mannitol				
1	EMB Aar- metallik sheen, MacConkey Aar- pink colored colonies	Gram negative, rod shaped bacilli	+	+	-	+	AG	ND	AG	AG	AG	<i>E. coli*</i> _α	7		30.43%
2	Nutrient Aar- golden yellow colored colonies	Gram positive clustered cocci	+					ND				<i>Staphylococcus</i> sp.	11		47.83%
3	Blood Aar- no hemolysis	Gram negative, coccobacillary	ND	-	+	+	AG	AG	NR	NR	AG	<i>Pasteurella</i> sp.*	3		13.04%
4	EMB Aar- no metallic sheen, MacConkey Aar- pink colore, mucoid colonies	Gram negative, short rod shaped bacilli	ND	ND	+	ND	AG	ND	AG	AG	AG	<i>Klebsiella</i> sp. _α	2	23	8.69%

MR= Methyl Red, VP= Voges-Proskauer, AG= Acid and Gas, +=Positive, -=Negative, ND=Not done, NR=No reaction

*= Mixed infection of *E. coli* and *Pasteurella* sp. from 2 cases

α= Mixed infection of *Klebsiella* and *E. coli* from 1 case



Fig.1. *E. coli* showing greenish colored colony with metallic sheen on EMB agar



Fig.2. *E. coli* in Gram staining showing Gram negative, short rod-shaped organism



Fig.3. *Staphylococcus* sp. produces yellow color colony on mannitol salt agar

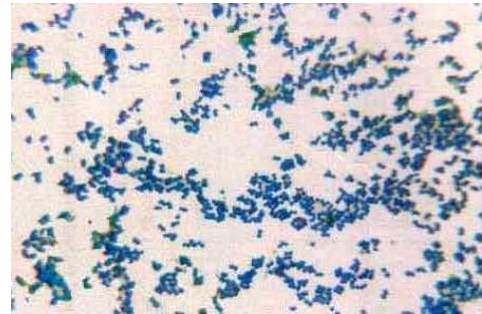


Fig.4. *Staphylococcus* sp. showing Gram positive, cocci and arranged in grape like clusters (Modified Gram staining $\times 830$)



Fig.5. *Pasteurella* sp. showing white, dull colony and no hemolysis on blood agar

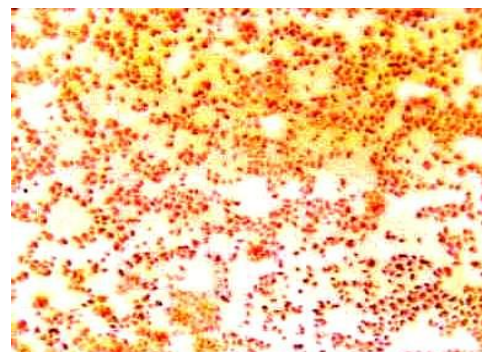


Fig.6. *Pasteurella* sp. showing Gram negative and coccobacillary shaped organism with bipolar appearance

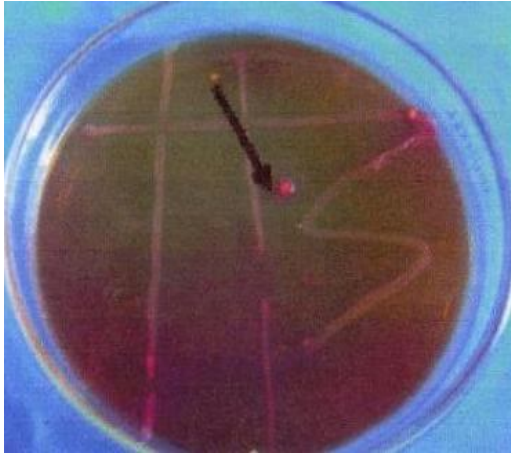


Fig.7. Pink colored mucoid colony produced by *Klebsiella* sp. on MacConkey agar

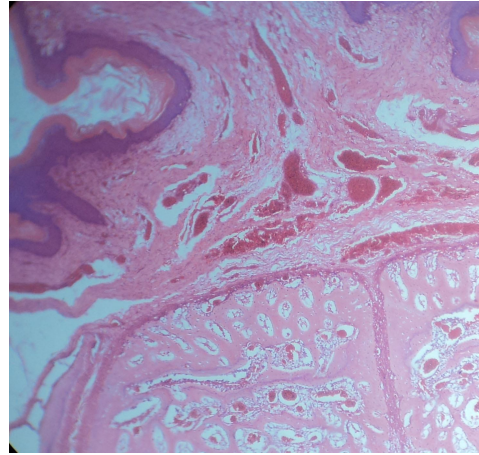


Fig. 8. The section of nasal passage showing acanthosis, mucous/parakeratosis, congestion of blood vessels and accumulation of inflammatory cells in case of *Pasteurella* sp. affected chicken. (H & E stain, ×333)

3.2. Colony characters, staining characters, and enzymatic activity tests of *Staphylococcus* sp.

In the present study 47.83% *Staphylococcus* sp. (Fig. 3) were found (11 *Staphylococcus* sp. in 23 isolates from 30 nasal passage swabs) in the nasal passages of dead chickens. These findings were lower than those reported by Trkylmaz (2005). The colonies of all isolates of *Staphylococcus* sp. on nutrient agar plate were golden yellow in color, they were gram-positive cocci arranged in a pairs or grape like clusters (Fig. 4) and catalase test positive which were reported by Brooks *et al.* (2002).

3.3. Colony characters, staining characters, biochemical tests and enzymatic activity test for *Pasteurella* sp.

The prevalence of *Pasteurella* sp. (Fig. 5) was 13.04% (3 *Pasteurella* sp. in 23 isolates from 30 nasal passage swabs) which was lower than the values reported by other authors (Murthy *et al.*, 2008; Trkylmaz, 2005). In this study the prevalence of *Pasteurella* sp. was less which might be due to age and breeds of the chickens

and also for the resistant power of the commercial chickens due to proper management, vaccination and nutrition. Whitish, opaque circular, translucent and non-hemolytic colonies on blood agar media produced by *Pasteurella* sp. isolates of present study which were similar to the findings of Chowdhury *et al.* (1985). In this study the staining characters of isolated *Pasteurella* sp. were coccobacillary, bipolar organisms in Leishman's staining (Fig. 6) and fermentation reaction of *Pasteurella* sp. with dextrose, sucrose and mannitol with production of acid only and no reaction occurred in lactose and maltose which were similar to the other authors (Prasad *et al.*, 1997; Chowdhury *et al.*, 1985).

3.4. Colony characters, staining characters and biochemical tests for *Klebsiella* sp.

The prevalence of *Klebsiella* sp. (Fig. 7) was 8.69% (2 *Klebsiella* sp. in 23 isolates from 30 nasal passage swabs) in the present study which was higher than the values reported by Popy *et al.* (2011) in Bangladesh. In that study the prevalence of *Klebsiella* sp. was less which

might be due to age and breeds of the chickens, geographic variation and also for the resistant power of the commercial chickens due to proper management, vaccination and nutrition. *Klebsiella* sp. isolates of this study produced pink colored mucoid colony on MacConkey agar, which was similar to the report of other author (Naowarat, 2007). The staining characters of isolated *Klebsiella* sp. were gram negative, thick rod shaped bacilli which corresponds with Deena and Adriana's (2005) findings. All *Klebsiella* sp. produced acid and gas in lactose, dextrose, maltose and mannitol. The organisms were Voges-Proskauer positive, methyl red negative and catalase positive which were also similar to the results of other authors (Naowarat, 2007; Deena and Adriana, 2005).

3.5. Colony characters of detected mixed infection

Two representative samples out of 30 were identified as mixed infection. One mixed infection comprised of *E. coli* and *Pasteurella* sp. while other mixed infection comprised of *Klebsiella* and *E. coli*. The colony characters, staining features and response to different biochemical and sugar fermentation tests of *Pasteurella* sp., *E. coli* and *Klebsiella* had already been discussed in previous section.

3.6. Gross pathology

Six tissue samples out of 30 cases showed gross lesions of nasal passage characterized by congested, hemorrhagic and mucous filled nasal passage, and erosion of passage tissue etc. Very similar findings were reported by many investigators (Esquinas and Iregui, 2007; Moustafa, 2005; Jaswinder *et al.*, 2005).

3.7. Histopathology

Histopathologically, no striking differences were found in tissues of nasal passage, though four types of bacteria were identified. The section of the nasal passages in general showed congested mucosa with excessive infiltration of inflammatory cells (Fig. 8). The lining epithelia

of nasal passage revealed acanthosis, hyperkeratosis and disruption of nasal epithelia. There was also hyperplasia of different mucous glands of nasal passages. However, on the basis of severity of lesions it seemed that *Klebsiella* sp. affected nasal tissues of chickens were severe than other bacteria affected nasal tissues of chickens. Almost same lesions of rhinitis caused by different bacteria were reported by many investigators (Esquinas and Iregui, 2007; Moustafa, 2005; Jaswinder *et al.*, 2005).

In this study, the bacteria isolated from the nasal passages were *E. coli*, *Pasteurella* sp., *Klebsiella* sp. and *Staphylococcus* sp. *E. coli* can cause different diseases in poultry including respiratory infection such as swollen head syndrome, respiratory rales, severe nasal discharge; *Pasteurella* sp is the etiological agent of fowl cholera and it also induces pneumonia, chronic rhinitis, facial edema; *Klebsiella* sp., *E. coli* and *Pasteurella* sp. combinedly cause pneumonia and tracheitis reported by several authors (Fuad and Mohammad, 2008; Moursi and Sabah, 2008; Drago and Don, 1996). However, in the present investigation, different kinds of lesions other than rhinitis were not found.

4. Conclusions

The recorded lesions of nasal passages of chicken in the present investigation were rhinitis. The prevalence of bacteria 30.43% *E. coli*, 47.83% *Staphylococcus* sp., 13.04%, *Pasteurella* sp., and 8.69% *Klebsiella* sp., were found in nasal passages of dead chickens. Two cases of mixed infection of *E. coli* and *Pasteurella* isolates, and one case of mixed infection with *Klebsiella* and *E. coli* isolates were identified. Grossly identifiable lesions such as congestion and mucus filled nasal passages were found. Microscopically, the section of the nasal passages in general showed congestion with excessive infiltration of heterophils and lymphocytes. The lining epithelia of nasal passage revealed acanthosis, hyperkeratosis and disruption of nasal epithelia. There was also hyperplasia of different mucous glands of nasal

passages. *Klebsiella* sp. affected nasal tissues showed comparatively severe lesions than other bacteria affected nasal tissues of chickens. The identified bacteria from nasal passage of chickens and pathological lesions will enrich the knowledge of veterinarian for the diagnosis of rhinitis and their treatment.

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