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Original article

Investigation on Infectious coryza of layer chicken in Bangladesh with isolation, identification and antibiogram study

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ABSTRACT

Among infectious diseases, infectious coryza is one of the major problems affecting commercial poultry industry in the developing country like Bangladesh. The present study was conducted for isolation, identification and characterization of *Haemophilus paragallinarum from* layer chicken in Bangladesh. A total of 122 samples (Nasal / tracheal swab, visceral organs like liver, lung, heart)) were collected from from Rangpur (Paragon Poultry Farm), Takhurgaon (North Egro Poultry Farm) and Dinajpur (Nizam Poultry Farm) districts of Bangladesh during the period from March 2011 to February, 2012 . The samples were collected from suspected birds based on age, sex, breed, temporal and spatial differences for the isolation and identification of *Haemophilus paragallinarum* by morphology, staining, cultural and biochemical properties. A total of 122 samples were screened by epidemiological study, of which the overall prevalence of *Haemophilus paragallinarum* was detected as 47.54 %. The prevalence was very high in laying hen (52.8%) and growing birds (42.8) in compare with the prelaying stage (16.6%). The prevalence of *Haemophilus paragallinarum* in Dinajpur, Rangpur and Thakurgaon were found 86.67%, 25%, and 34.21% respectively. The isolates were resistant to norfloxacin and tylosin. It was evident that

amoxycillin and gentamicin can be of better value in the treatment of infectious coryza in layer chickens in Bangladesh.

1. Introduction

Infectious Coryza (IC) is an infectious contagious respiratory bacterial disease of several avian species and the etiological agent responsible for the diseases is *Haemophilus paragallinarum*. The disease at initial stages may be acute to sub acute but progresses to a chronic state as the disease works through the flock. Common names for the disease are roup, cold and Coryza (Blackall *et al.,* 1997). The clinical syndrome was first diagnosed in 1931 by De Blieck. Since the disease proved to be infectious and primarily affected nasal passages, the name "infectious coryza" was adopted (Blackall and Yamamoto, 1989). Coryza is a disease of the upper respiratory tract- trachea, sinuses and air passages of the head. Disease is characterized by nasal discharge, facial swelling, sneezing, labored breathing and fetid odor of the exudates. The disease occurs worldwide and the reasons behind success of survival for this bacterium is that after recovering from infection, birds become carriers, therefore aiding the spread of *H. paragallinarum*. Secondly, the bacterial strain belongs to one of nine serovars, which makes combating the spread of the disease through inactivated vaccination ineffective especially due to low cross protection among these serovars (Rimler et al., 1977). Due to the phenomenon that the disease proved to be infectious only in the nasal passages the name "Infectious Coryza" was adopted. Involvement of the lower respiratory tract may be due to synergism between *H. paragallinarum* and other respiratory tract pathogens (Blackall and Yamamoto, 1989). Economic magnitude of the disease is due to its effect in both broiler and layer birds. Egg production in affected laying flocks may drop 10-40 per cent. Affected birds have severe respiratory difficulties resulting in 2 per cent to more than 10 per cent mortality. Young birds grow poorly and hence, there is loss of condition in broiler, ultimately resulting in increased number of culls.

In Bangladesh no documented study has been reported considering isolation , characterization and control of this remedy although this problem has become a constant threat to our poultry industry because of its frequent occurrences at farmers level. Related works were done in Great Britain, Australia. Japan, USA, Africa, Indonesia, Morocco, China, Thailand, California including neighboring countries of the Bangladesh since 1989. In our country, the disease is usually controlled by antimicrobial drugs and also by using the imported vaccine. In case of antibiotics used to control the disease and also the imported vaccine results drug resistance and might lead to less efficacious against our local isolates of *Avibacterium paragallinarum* organism. To prevent the economic losses, it is necessary to isolate & characterize the etiological agent of this disease and to develop the remedial measures to this serious poultry threat.

2. Materials and methods

2.1. Study area

The present research work was carried out on layer chickens of Bangladesh during the period from March, 2011 to February, 2012 at the Bactreiology laboratory of the department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

2.2. Collection of data and samples

A total of 122 samples (Nasal / tracheal swab, visceral organs like liver, lung, heart)) were collected from from Rangpur (Paragon Poultry Farm), Takhurgaon (North Egro Poultry Farm), Dinajpur (Nizam Poultry Farm) districts of Bangladesh during the period from March 2011 to February, 2012 . The samples were collected from suspected birds based on age, sex, breed, temporal and spatial differences for the isolation and identification of bacterial pathogen by morphology, staining, cultural and biochemical properties. Precautions were taken to avoid contamination of one sample with other. The date of collection, age, sex, breed, clinical signs and environmental history were recorded for each case. The birds were divided into three groups as group - A (0-9 weeks old), group

– B (10-20 weeks old) and group - C (above 20 weeks old).The isolated bacteria were tested for the sensitivity pattern to different antibiotics.

2.3. Isolation and identification of causal agent

2.3.1. Cultural characterization

Isolation of bacterial pathogen from suspected samples were carried out by culturing the samples on blood agar and chocolate agar plate cross streaked with *Staphylococcus* spp. The inoculated plates are then incubated at 37◦ C with 5 -10 % CO_2 for 24 - 48 hours. Identification of the bacterial agent from the pure culture were carried out based on their colony characteristics, satellitism phenomenon, and hemolysis pattern as described by (Blackall, *et al*., 1997) and (Chen *et al*., 1998).

2.3.2. Morphological characterization

The colonies from pure culture were then studied for its morphological characters by Gram's stain described by Buxton and Fraser, 1977.

2.3.3. Biochemical characterization

Different biochemical tests were employed to the organism like different Sugar's fermentation, Indol production, Voges-Proskauer, methyl red, Hydrogen sulphide production and nitrate reduction tests to confirm the pathogen as *Haemophilus paragallinarum*.

2.3.4. Antibiotic sensitivity test

In vitro antibiotic sensitivity test of isolated *Haemophilus paragallinarum* was performed with the standardized commercial sensitivity discs manufactured by Oxoid limited (Basingstoke, Hampshire, England).Among the isolated bacterial agent representative isolate were subjected to sensitivity test by disc diffusion test according to the method described by Bauer *et al*.(1966).This method allowed the rapid determination of in vitro efficacy of an antibiotic by measuring the diameter of the zone of inhibition, which results from diffusion of the agent into the medium surrounding the disc.

2.4. Maintenance of stock culture

During the experiment it was necessary to preserve the isolated organisms for longer periods. For this purpose, pure culture of the isolated organisms was stored in 20% sterilized glycerin and sealed with paraffin wax and stored at - 80°C in freezer for future use.

3. Results

A total of 122 samples were screened by epidemiological study, of which the overall prevalence of *Haemophilus paragallinarum* was detected as 47.54 % (Table 1). The prevalence was varied in terms of age (Table 1). It indicated that prevalence is very high in laying hen (52.8%) and growing birds (42.8) in compare with the prelaying stage (16.6%).

Table 1
Prevalence of *Haemophilus paragallinarum* detected from suspected birds based on age, sex and breed.

Group of birds (Wks)	Total No. of birds in flock	Name of the sample	No sample tested	Positive case	% of Positive case	Overall Prevalence (%)
A = 0-9	2000	Nasal/	21	09	42.8	
B = 10 -20	3000	Tracheal	12	02	16.6	
C = ≥20	1200	swab	89	47	52.8	47.54
Grand total			122	58	47.54	

The prevalence of *Haemophilus paragallinarum* in Dinajpur, Rangpur and Thakurgaon were found 86.67%, 25%, and 34.21% respectively (Table 2). It has been observed that the prevalence of Infectious Coryza in chicken

showed significant variation in spatial differences.The highest prevalence was found in Dinajpur (86.67%) and the lowest in Rangpur (25%). These differences may be due to several factors such as geoclimatic situation, passive immunity level, infecting dose, simultaneous infection with other respiratory pathogens, stress, management errors and biosecurity failures.

In this study, 58 samples were found positive for *Haemophilus paragallinarum.* The association of Infectious Coryza with different seasons (Table 2) revealed that higher prevalence of Infectious Coryza was found in winter season (48.63%) and in summer season (5.26%). The present study detected the highest percentage (48.63%) of Infectious Coryza in layer chicken at winter season.

The birds in the infected flock had facial swelling, nasal and lacrimal discharge, open mouth breathing and mucoid discharge from the nares. The clinical signs are common features of infectious coryza. The bacterium was recovered only from nares on Chocolate Agar and Blood Agar (Table 3). No growth was recovered from samples like liver, lungs, heart streaked on different agars. The growth and morphology characteristics indicated that the organism isolated might be one of *Haemophilus* species (Table 4), which was later confirmed by different biochemical tests (Table 5).

The results of antimicrobial susceptibility of the isolated *Haemophilus paragallinarum* were summarized in Table 6. Out of 10 isolates, 10 were susceptible to amoxycillin and gentamicin. On the other hand, out of 10 isolates, 100% isolates were resistant to tylosin and norfloxacin. Furthermore, 80% isolates were susceptible to erythromycin, sulphamethoxazole-trimethoprim and oxytetracycline. Moreover, 40% isolates were resistant to enrofloxacin.

Table 2
Demonstration overall prevalence of isolated *Haemophilus paragallinarum* by temporal and spatial differences.

Name of District	Season					
	Winter			Summer		
	Total no. sample tested	No of positive isolates (%)	Overall Prevalence (%)	Total no. sample tested	No of positive case	Prevalence of positive isolates (%)
Dinajpur (56)	45	39 (86.67)	48.63	11	1(9.09)	5.26
Thakurgaon (44)	38	13 (34.21)		6	0	
Rangpur (22)	20	5 (25)		2	0	
Total	103	57		19	1	

Table 3
Determination of 'V 'factor for the growth of *Haemophilus paragallinarum* by *Staphylococcus* spp.

Name of the media	Colony characteristics
Blood Agar Chocolate Agar	Small ,dew drop like nonhemolytic colonies
Chocolate Agar Cross streaked with *Staphylococcus sp.*	Luxuriant growth

4. Discussion

In this study, the overall prevalence of Infectious Coryza was 47.54% by age, sex and breed of birds in layer chicken of Bangladesh. The prevalence was higher in laying hen (52.8%) in compare with growing (42.8%) and prelaying stage (16.6%). This findings support the earlier observation of Blackall *et al.,* (1997).This increased prevalence of infectious coryza in laying hens might be due to increased length of exposure of laying hens to pathogens compared to grower and prelaying stage. The prevalence of infectious coryza was influenced by the temporal and spatial differences (Terzolo *et al.,* 1993 and Blackall *et al.,* 1997).In the present study a trend in increase in the rate of prevalence of infectious coryza was observed as the location and seasonal variations. The association of infectious coryza with different seasons revealed higher rate of prevalence in winter season

(48.63%) comparing with summer season (5.26%). Our present findings support the earlier observation of Chen *et al.,* 1993 and Blackall *et al.,* 1997.

In our present study it also observed that the prevalence of infectious coryza in layer chicken showed significant variation in location differences.The higher rate of prevalence of infectious coryza in layer chicken was found in Dinajpur (86.67%) in compare with Thakurgaon (34.21%) and Rangpur (25%) reapectively.This observed variation in the prevalence of infectious coryza in various areas of Bangladesh could be related with several factors such as geoclimatic situation, passive immunity level, infecting dose, simultaneous infection with other respiratory pathogens, stress managemental practice, biosecurity failure and different locations of the study areas. In the present study, the birds of infected flock had facial swelling, nasal and lacrimal discharge, open mouth breathing and mucoid discharge from the nares that are the common features of infectious coryza in chicken. Our present findings support the earlier observations of (Schalm & Beach, 1936, Droual *et al*., 1990, Horner *et al*., 1992, Mouahid *et al*., 1992, Calnek *et al*., 1991 and Sandovel *et al*., 1994).

Table 4
Chracterization of field isolates of *Haemophilus paragallinarum* by morphological, staining, cultural and biochemical examination.

No. of tested isolates	Test performed	Observation	Response		Indication
			Positive isolates	% of Positive isolates	
122	Microscopic examination by Grams staining	Showing Gram negative, coccobacilli shape	58	100	*Haemophilus paragallinarum*
	TSI agar slant reaction	Ferment Glucose , Sucrose & Lactose	58	100	*Haemophilus paragallinarum*
	Motility test by MIU medium	Absence of turbidity	58	100	*Haemophilus paragallinarum*
	Indole test	No pink color ring at the adjacent	58	100	*Haemophilus paragallinarum*
	MR test	Absence red color indicate MR test negative	58	100	*Haemophilus paragallinarum*
	VP test	No color change indicate VP test negative	58	100	*Haemophilus paragallinarum*
	H_2S Production	Absence of black coloration at TSI slant indicate H_2S Production negative	58	100	*Haemophilus paragallinarum*

In the present study, the bacterium was isolated from nares on chocolate aga and blood agar cross streaked with a nursery colony of *Staphylococcus aureus* as feeders. In our present study it was observed that satellitic growth patterns of isolated bacterium might be one of *Haemopilus s*pecies, which was later confirmed by biochemical tests. This observation supported by similar results found by other researchers (Tobias *et al.,*2001,

Terzolo *et al.,* 1993, Rimler *et al.,*1976 and Miflin *et al.,*1995). In this study, the isolated organism was characterized by morphological characterization (Gram's staining) and different biochemical tests. This observation revealed that the isolated organism was Gram negative, short rods or cocccobacilli arranged in single or pairs. This finding was supported by (Tobias *et al.,* 2001, Terzolo *et al.,* 1993, Rimler *et al.,* 1976 and Miflin *et al.,* 1995). In our present study, it was observed that caseopurulent air sac lesions in field cases of infectious coryza in chicken. This observation was supported by Blackall *et al.,* 1989 and Rimler *et al.,* 1976.

Table 5
Biochemical reactions of the isolate

Test	Result
Glucose	+
Sucrose	+
Lactose	+
Indol	-
Vogas Proskauer test	-
Methyl Red test	-
H_2S Production	-
Motility	-

Table 6
Antimicrobial susceptibility pattern of the isolated *Haemophilus paragallinarum* (n = 10).

Antimicrobial agent	No (%) of *Haemophilus paragallinarum*		
	Susceptible	Intermediate	Resistant
Amoxycillin	10 (100)	0 (0)	0 (0)
Erythromycin	8 (80)	0 (0)	2 (20)
Gentamicin	10 (100)	0 (0)	0 (0)
Enrofloxacin	6 (60)	0 (0)	4 (40)
Sulphamethoxazole-Trimethoprim	8 (80)	0 (0)	2 (20)
Oxytetracycline	8 (80)	0 (0)	2 (20)
Tylosin	0 (0)	0 (0)	10 (100)
Norfloxacin	0 (0)	0 (0)	10 (100)

In the present study, the antibiogram study revealed that all of the field isolates were sensitive to various antibiotics used in this study at varying levels. All the tested isolate were found highly sensitive to amoxycillin, oxytetracycline and gentamycin suggesting that these antibiotics could be the first choice of drug for the treatment purpose. These results are partially similar to the findings of Rajurkar *et al.* (2010). The antimicrobial agents are of great value for devising curative measures against bacterial infections. Use of antimicrobials in livestock production is suspected to significantly contribute to multiple drug resistance in species of bacteria, which are common to humans and animals (Acar and Rostel, 2001).

5. Conclusion

The prevalence of infectious coryza in layer chicken of Bangladesh was 47.54%. Certain risk factors such as age, breed, geoclimatic situation, and stress, other respiratory pathogens and managemental practice associated with field cases of layer chicken in Bangladesh influenced the prevalence of infectious coryza in chicken. Our present findings suggest that application of broad spectrum antibiotic could be an effective way to control the disease with some modification in the farm management system but it does not eliminate the carrier status of chickens. It is advisable to vaccinate the chickens with inactivated coryza vaccine to prevent economic losses. Considering this fact the research work will also extends for the production of vaccine candidate from the field isolate to control infectious coryza in layer chicken of Bangladesh.

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