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Prevalence of Colibacillosis in birds in and around Guwahati city (Assam)

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Abstract
During the period of this investigation from July 2016 to June 2017, a total of 268 carcasses of birds irrespective of the age, breed and sex were collected from organized and unorganized farms in and around Guwahati city. In bacteriological isolation of *Escherichia coli* using MacConkey agar (MLA) and Eosin methylene blue (EMB), 46 cases were found positive for colibacillosis. The overall prevalence of colibacillosis was recorded as 17.16%. The different pathological conditions results from colibacillosis observed in the present study were colisepticaemia (n=23), enteritis (n=13), oophoritis (n=8) and omphalitis (n=2). On the basis of age group, the highest prevalence of colibacillosis was recorded at the age group of 3-6 weeks compared to the other age groups. Again highest prevalence of colibacillosis in urban and semi-urban areas were recorded at 38.46 percent and 50 percent respectively in 3 to 6 weeks of age group.

Keywords: Colibacillosis, Diagnosis, Isolation, Prevalence

1. Introduction

India is an agriculture based country where farmers mostly engage themselves in agriculture and animal husbandry practices. Poultry industry in India is considered to be the fastest growing industry among the agriculture based industries. In India there are 20 indigenous breeds of chickens with some special characteristics [1]. In Assam backyard poultry farming is very popular in rural areas. Almost every household of Assam keeps poultry as their secondary livelihood. The productivity of indigenous chicken is very low in comparison to commercial broilers and layers. The low productivity is due to genetic makeup and mortality due to mismanagement, diseases, lack of balanced ration and predators. Higher mortality in poultry is found to be due to outbreak of different infectious diseases [2].

The poultry industry in India as a whole is facing some problems due to affection of some infectious and non-infectious diseases. Colibacillosis is considered as one of the principal cause of morbidity and mortality in birds and associated with heavy economic losses to the poultry industry with its association with various disease conditions either as primary pathogen or as a secondary pathogen [3]. Colibacillosis is a group of diseases that include yolk sac infection/omphalitis/mushy chick disease, colisepticaemia, coligranuloma, egg peritonitis, polyserositis, salpingitis, panopthalmitis, cellulitis etc. In acute form of the disease is characterized by septicaemia resulting into death and in the subacute form there is pericarditis, airsacculitis and perihepatitis [4, 5].

Colibacillosis is a bacterial disease caused by *Escherichia coli,* a gram negative, rod shaped facultative anaerobic bacterium under the family *Enterobacteriacae,* the organism was first described by Theodor Escherich, a German-Austrian pediatrician in 1885. Colibacillosis occurs in all age groups of birds. Faeco-oral route is the main route of infection followed by ingestion of contaminated feed and water. Intestinal tract of animals including poultry is the most important reservoir site of *E.coli.* Transmission of avian pathogenic *E. coli* (APEC) through the egg is a common route and may cause high mortality in chicks. Pathogenic coliforms are more frequent in the gut of newly hatched chicks than in the eggs from where they hatched, suggesting rapid spread after hatching [4, 6]. In broilers, the prominent clinical manifestations of the disease were depression, respiratory distress and retardation of growth. Morbidity may reach upto 50%, but mortality remains below 5% [7].

There are several reports of colibacillosis from different parts of India reported by different workers [8, 9, 10]. In last few years, large percentage of chick mortality was also recorded from different farms within or outside of Guwahati city. Therefore this study was undertaken to

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study the diagnosis and prevalence of colibacillosis in and around Guwahati city.

2. Materials and Methods
The study was carried out during the period from July 2016 to June 2017. Materials were collected from the organized and unorganized farms in and around Guwahati city.

2.1 Sample collections
For bacteriological examination, representative tissue samples showing gross lesions of colibacillosis at the time of necropsy examination were collected aseptically in sterilized containers. The tissue samples collected were heart, liver, kidneys, lungs, intestine, ovary etc. Heart blood was also collected for culture of *E. coli.*

2.2 Bacteriological examination
2.2.1 Bacterial isolation and idetification
The samples were directly inoculated into MacConkey agar (MLA) and incubated at 37 ^{0}C for 24 hours. Lactose fermenting pink coloured colonies in MLA suspected for *E.coli* were further subculture on Eosin methylene blue (EMB) agar and incubated at 37 ^{0}C for 24 hours to obtain pure cultures of *E.coli.* Identification and confirmation of the isolates were done on the basis of morphology, colony characterstic, staining characterstic and biochemical reaction as per previously described procedure [11, 12].

2.2.2 Gram's-staining and biochemical tests
Gram's-staining was done using standard procedure where organisms were visible as pink colour colonies. Different biochemical tests used for identification of the bacteria were sugar fermentation, methyl red, Voges-Proskauer and Indole production tests [13].

2.3 Prevalence study
The overall prevalence of colibacillosis in and around Guwahati city was studied based on bacteriological examinations. Prevalence of colibacillosis was also studied separately in urban and semi-urban areas.

2.3.1 Age-wise prevalence study
To study the age wise prevalence of colibacillosis, total birds were categorized into five age groups viz. 0-3 weeks, 3-6

weeks, 6-9 weeks, 9-12 weeks and above 12 weeks. Prevalence of colibacillosis in different age groups was also studied in urban as well as in semi-urban areas.

3. Statistical analysis
All the data were analyzed by using standard software (IBM SPSS Statistics 20) and results were expressed in percentage.

4. Results and Discussions
4.1 Prevalence of colibacillosis
In the present study from July 2016 to June 2017 altogether 268 carcasses of birds were collected from organized and unorganized farms located in and around Guwahati. Carcasses were collected irrespective of the age, breed and sex. The carcasses of birds were necropsied in the Department of Veterinary Pathology, College of Veterinary Science, Khanapara, Guwahati. Out of 268 carcasses 180 birds were suspected for colibacillosis. After bacterial isolation 46 cases of birds were confirmed for colibacillosis. The overall prevalence of colibacillosis was 17.16% (Table 1). Different prevalence rate of colibacillosis were also recorded by earlier workers [14, 15, 16, 17, 18, 19] in various parts of the world. The pathological conditions of colibacillosis observed in the present study were colisepticaemia (n=23), enteritis (n=13), oophoritis (n=8) and omphalitis (n=2). The variation in the prevalence rate might be due to different managemental and geo-climatic conditions.

4.1.1 Prevalence of colibacillosis on the basis of age of the birds
On the basis of age group the highest prevalence of colibacillosis during this study was in the age group of 3-6 weeks (42.50%) and the lowest was in the age group of 9-12 weeks (14.28%) irrespective of urban and semi urban areas (Table 1). Highest prevalence in 3-6 months of age were also observed by many previous workers [9, 15, 20]. However, some workers reported higher prevalence in the age group of 0-2 weeks but minimum at the age group of >4 weeks [21]. Gradual reduction of maternal immunity along with the age may be the cause of the higher incidence of colibacillosis in 3-6 weeks of age. Frequent exposure of *E. coli* may enhance the immunity in the later stage of life reducing the prevalence of colibacillosis.

Table 1: Prevalence of colibacillosis on the basis of age of the birds

No. of birds necropsied	No. of suspected cases/confirmed cases	Age group (weeks)					Prevalence
		0-3	3-6	6-9	9-12	>12	
268	180/46	49/14 28.57%	40/17 42.50%	32/6 18.75%	21/3 14.28%	38/6 15.78%	17.16%

4.1.2 Prevalence of colibacillosis in urban and semi urban area
The prevalence of colibacillosis was higher in urban areas than in semi urban areas of Guwahati, which was found at 16.67% (Table 2) and 18.29% (Table 3) respectively. In urban areas, highest prevalence of the disease was observed in 3-6

month (38.46%) age group and least in >12 months (15.79%) age group (Table 2). Similarly, in semi urban areas also age between 3-6 months (50%) showed highest prevalence compared to other age groups and 9-12 months and >12 months age groups revealed zero prevalence (Table 3). There was no sufficient published literature to support this finding.

Table 2: Prevalence of colibacillosis on the basis of age of the birds (a) in urban areas

No. of birds necropsied	No. of suspected cases/ confirmed cases	Age group (weeks)					Prevalence
		0-3	3-6	6-9	9-12	>12	
186	129/31	29/8 27.58%	26/10 38.46%	20/4 20.00%	16/3 18.75%	38/6 15.79%	16.67%

Table 3: Prevalence of colibacillosis on the basis of age of the birds (b) in semi urban areas

No. of birds necropsied	No. of suspected cases/ confirmed cases	Age group (weeks)					Prevalence
		0-3	3-6	6-9	9-12	>12	
82	51/15	20/6 30.00%	14/7 50.00%	12/2 16.6%	5/0 00.00%	0/0 00.00%	18.29%

4.2 bacteriological examination
In this Investigation on the basis of morphology, colony characteristics, staining characteristic and biochemical reactions a total of 46 cases *E. coli* were isolated and identified. A total of 93 isolates of *E. coli* was recovered from different organs of the affected birds.

4.2.1 Isolation and Cultural characteristic of *E. coli*
In this investigation the *E.coli* organism was isolated by using MacCokey agar media and Eosin methylene blue (EMB) agar media. The cultural characterstic of the organism in MacConkey agar showed pink colour colonies and Metallic sheen in the EMB agar (Fig. 1&2). Similar findings were reported by previous workers [18, 21, 22].

4.3 Biochemical test
4.3.1 IMVIC test
In IMVIC test all the *E.coli* isolates showed a positive reaction for Indole test and methyl red test, but negative for Voges Proskauer test and citrate utilization test (Fig. 3). These findings support the findings of several workers [22, 23].
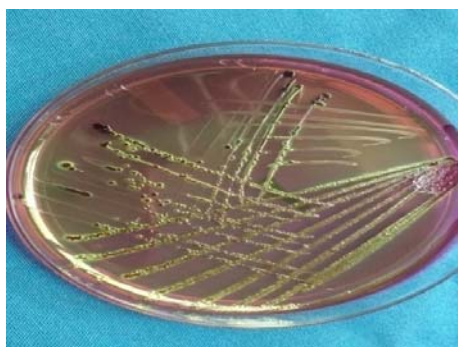
4.3.2 Sugar fermentation test
All the isolates fermented glucose, lactose, mannitol, arabinose and sorbitol. These findings support the findings of previous workers [22, 24].
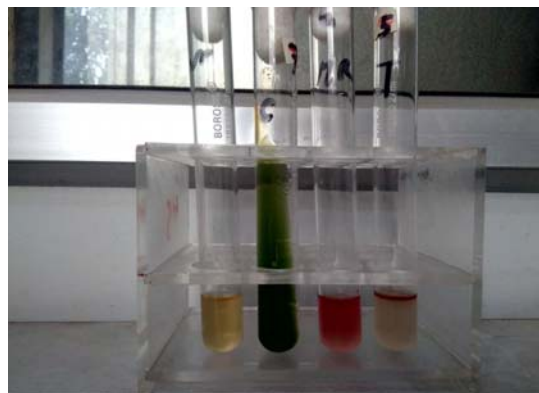
4.4 Gram's stain
In Gram's stain the organisms appeared as pink coloured, rods remains singly or in pairs.
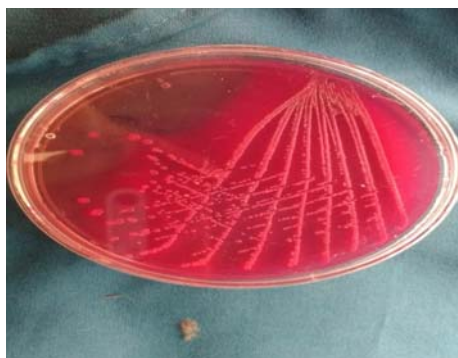
Fig 1: *E.coli* showing pink colour colonies in MacConkey agar

Fig 2: *E.coli* colonies showing metallic sheen in EMB agar

Fig 3: IMVIC reaction test

5. Conclusions
The prevalence percent of colibacillosis recorded during this investigation was 17.16%. On the basis of age groups the highest prevalence of colibacillosis was recorded at the age group of 3-6 weeks compared to the other age groups. Different pathological conditions results from colibacillosis observed in this study were coliseptisemia, enteritis, oophoritis and omphalities. Age wise prevalence of colibacillosis in urban and semi-urban areas were also recorded at 38.46 percent and 50 percent respectively in 3 to 6 weeks of age groups.

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7. References
1. Sharma RP, Chatterjee RN, Niranjan M. Poultry production under backyard system: Improvement approaches. In: National symposium on conservation and improvement of animal genetic resources under low input system; challenges and strategies, NBAGR, Karnal. 2006, 72-77.
2. Lalrinhui. Etiopathological studies on mortality in Vanaraja breed and indigenous fowl maintained under deep litter system of management, M.V.Sc.Thesis, Assam Agricultural University, Khanapara, Guwahati, 2012, 22.
3. Barnes JH, Vaillancourt J, Gross WB. Colibacillosis. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM and McDougald LR (Ed.). Diseases of poultry. Ed, 11, Iowa University Press, Ames, IA, 2003, 631-652.
4. Dhama K, Chakraborty S, Barathidasan R, Tiwari R, Rajagopalan S, Singh SD. Escherichia coli, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance: A review. Res. Opin. Anim. Vet. Sci. 2013; 3(6):179-194.
5. Dadheech T, Vyas R, Rastogi V. Prevalence, Bacteriology, Pathogenesis and Isolation of E.coli in Sick Layer Chickens in Ajmer Region of Rajasthan, India, International Journal of Current Microbiology and Applied Sciences, 2016; 5(3):129-136.

6. Adesiyum K, Offiah N, Seepersadsingh N, Rodrigo S, Lashley V, Musal L *et al.* Microbial health risk posed by table eggs in Trinidad. Epidemiol. Infect. 2005; 133:1049-1056.
7. Vandekerchove D, De Herdt P, Lacvens H, Pasmans F. Colibacillosis in battery- caged layer hens: clinical and bacteriological characterstics and risk factor analysis. Avian Pathology. 2004; 33(2):117–125.
8. Kumar A, Jindal N, Shukla CL, Ansari RK, Leudox DR, Rottinghaus DR. Pathological changes in broiler chickens fed ochratoxin A and inoculated with Escherichia coli. Avian pathology. 2004; 31(4):413-417.
9. Ashwin KD, Gupta PRB, Kumari D. Pathological studies on natural cases of avian colibacillosis in Haryana state. Haryana Vet. 2013; 52:118–120.
10. Srinivasan P, Balasubramanium AG, Murthy KGRT, Balachandra P. Prevalence and Pathology of egg bound syndromes in commercial white leghorn chicken, J. World's poult. Res. 2014; 4(2):30-36.
11. Cruickshank R, Duguid JP, Marimion BP, Swoin RHA. Medical Microbiology, 12th edn. Vol. ii, Churchill Livingstone, Edinburgh, London and New York.
12. Edwards PR, Ewing WH. Identification of enterobacteriacae. Int. J. Syst. Bacteriol. 1986; 36:581-582.
13. Cheesbrough M. Medical Laboratory Manual for Tropical Countries, Microbiology. 1st ed. London: English Language Book Society; 1985, 400-480.
14. Lalrintluanga C. Incidence and etio-pathology of broiler mortality in Assam. M.V.Sc. Thesis. 1991; Assam Agricultural University, Khanapara, Guwahati-22.
15. Shah QA, Soomro NM, Tunio SN. Colibacillosis in broiler: prevalence and pathology. One line Journal of Biological Sciences. 2003; 3(3):287-290.
16. Bania R. Prevalence and etiopathology of mortality in Kalinga brown breed of poultry in Assam. M.V.Sc. Thesis. 2009; Assam Agricultural University, Khanapara, Guwahati-22.
17. Buragohain R, Kalita G. Assessment of mortality pattern of broiler under intensive system of management in Mizoram. Tamilnadu J. Veterinary & Animal Sciences. 2010; 6(5):239-241.
18. Hasan R, Ali MH, Siddique MP, Rahman MM, Islam MA. Clinical and laboratory diagnosis of common bacterial diseases of broiler and layer chickens. Bengl. Vet. Med. 2010; 8(2):107–115.
19. Badruzzaman ATM, Noor M, Mamun MA, Husna A, Islam KM, Alam KJ *et al.* Prevalence of diseases in commercial chickens at Sylhet division of Bangledesh. International Clinical Pathology Journal. 2015; 1(5):00023.
20. Uddin MB, Ahmed SSU, Hassan MM, Khan SA, Mamun MA. Prevalence of poultry diseases at Narsingdi, Bangladesh. Int. BioRes. 2010; 1(6):09-13.
21. Hashem MA, Elahi MF, Mannan MA, Kabir MHB, Kashem MA, Pallab MS. Isolation, identification and antibiogram of *Escherichia coli* from broiler at Chittagong district in Bangladesh. Wayamba Journal of Animal Science. 2012; 578X:312-316.
22. Tonu SN, Sufian AM, Kamal MM, Rahman HM, Hossain MM. Pathological study on colibacillosis in chickens and detection of *Escherichia coli* by PCR, Bangl. J. Med. 2011; 9(1):17-25.
23. Daud NHA, Htin NN, Paan FH, Kyaw T, Khaing AT, Abba Y *et al.* An outbreak of colibacillosis in broiler farm. Journal of Animal and Veterinary Advances. 2014; 13(8):545-548.
24. Khaton R, Haider MG, Paul PK, Das PM, Hossain MM. Colibacillosis in commercial chickens in Bangladesh. The Bangladesh Veterinarian. 2008; 25(1):17-24.