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Serotyping and prevalence of avian pathogenic *Escherichia coli* infection in broilers in Kashmir

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Abstract
Among poultry diseases, which cause huge economic loss in terms of mortality and condemnation of carcasses at slaughter houses, avian colibacillosis caused by *Escherichia coli (E. coli)*, is considered as one of the major and principal causes of morbidity and mortality. Present Investigation was aimed at studying the prevalence of colibacillosis in commercial broiler chicken and to identify the most predominant serotype of *E. coli* in broiler chicken. Samples comprised of mortalities from various poultry farms operating in Srinagar and Ganderbal district along with their adjoining areas. Colibacillosis constituted significant component of mortality among broiler chicken of all age groups ranging from 23.634% to 29.845% with overall mean of 26.357%. The most prevalent serogroup of *E. coli* observed was O76 (15.59 %), followed by 14.45 % O8, 12.17 % O1, 7.22 % O26, 6.44 % O20, 4.94 % O114, 4.18% O11, 3.80 % O2, and 3.04 % each O45 and O84. However, 15.21% untypeable, and 6.08% rough type were also observed. Impression smears revealed Gram negative, capsulated and cocobacillary organisms. The cultural and biochemical characteristics were characteristic of *E. coli.*

Keywords: serotyping, prevalence, APEC, *Escherichia coli*

Introduction
Poultry rearing in India was mostly a backyard activity prior to 1960's being in its juvenile stages. However, during the last four decades, backyard poultry farming has enormously shifted towards commercialization. Jammu and Kashmir State has also shown a positive trend in poultry production with a rise from 2.03 million (1970) to 6.68 million (Livestock census 2007). The poultry production has contributed 318.6 crore to State's economy by producing 236 lac kg of poultry meat in 2010-2011(JK Animal husbandry.net) which is much higher as compared to production in 2001 (40 lac kg of meat worth 16 crores). In recent years, exploitation of avian genetic resources by selective breeding has led to evolution of superior strains of broiler chickens. However, the changing structure of management, poor nutrition and many other factors like sudden climatic changes have imposed stress on poultry birds making them more vulnerable to diseases. The scenario of poultry diseases has also changed with emerging and re-emerging disease flaring up, thus imposing threat to the poultry industry. Among poultry diseases, which cause huge economic loss in terms of mortality and condemnation of carcasses at slaughter houses, avian colibacillosis caused by *Escherichia coli*, is considered as one of the major and principal causes of morbidity and mortality either as primary pathogen or as a secondary pathogen [1]. Natural outbreak of colibacillosis in young chicken have been reported but concurrent course of illness among chicks of different age groups is quite intriguing, especially in view of multiplicity of different serogroups having different virulence capacity. Hence this work was under taken to study the prevalence of colibacillosis in commercial broiler chickens and to identify the most predominant serotype of *Escherichia coli* in broiler chickens in Jammu And Kashmir State.

Materials and Methods
Sampling
Samples comprised of mortalities from various poultry farms operating in Srinagar and Ganderbal district along with their adjoining areas and those which were brought to Division of Veterinary Pathology for post-mortem examination. The outbreaks suspected for *Escherichia coli* in broiler chicken were identified based on the history, clinical signs and lesions, after following a thorough post mortem examination of birds. History of each suspected flock

was recorded which included flock size, mortality and total number of birds per outbreak.

Isolation and Identification

Representative samples (heart, spleen, lung, liver, ceaca, bursa etc) were inoculated into nutrient broth and incubated at 37ºC for 24 hours. The bacterial growth in the nutrient broth was re-inoculated on MacConkey agar plates (HiMedia, Mumbai, India) and the plates were incubated at 37ºC for 24 hours. The lactose fermenting colonies on MacConkey plates were re-inoculated on Eosin Methylene Blue agar (HiMedia, Mumbai, India). The *Escherichia coli* colonies typically showing metallic sheen were transferred to the nutrient agar slants and stored at 4°C for further characterization. Identification of isolates was further carried out using standard morphological and biochemical tests including Grams staining and IMViC tests.

Sero grouping

The *Escherichia coli* isolates which were characterized in house by standard morphological and biochemical tests were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli-173204 (H.P), for sero grouping.

Results

Prevalence of Colibacillosis in Broiler Chicken

The overall mortality and prevalence of colibacillosis among broilers of different age groups is given in Table 1. A total of 118 outbreaks were recorded in broiler chicken of different age groups which included 20, 23, 30, 25 and 20 outbreaks in age groups of 0-7, 8- 14, 15- 21, 22- 28 and above 29 days of age respectively. The overall mortality in the flocks was 3.098%. with highest mortality of 4.627% (1021/22064) recorded in the age group of 8- 14 days, followed by 3.397% (1223/36007) in the age group of 15- 21 days, 3.280% (952/29025) in age group of 0-7 days, 2.661% (652/24500) in age group of 22- 28 days and 1.126% (223/19800) in the age group of above than 29 days.

Out of a total number of 4071 carcasses necropsied colibacillosis was observed in 1073 cases giving a case prevalence of 26.357%. The case prevalence of colibacillosis in different age groups was comparable with highest of 29.845% (365/1223) in 15- 21 days of age, followed by 26.457% (59/223) in age group of more than 29 days, 25.767% (168/652) in age group of 22 -28 days, 25.073% (256/1021) in age group of 8 – 14 days and 23.634% (225/952) in age group of 0 – 7 days. The proportionate mortality due to colibacillosis was 24.016% (365/1073), 23.858% (256/1073), 20.969% (225/1073), 15.657% (168/1073) and 5.498% (59/1073) respectively in the age groups of 15- 21, 8- 14, 0- 7, 22 -28, and more than 29 days of age in that order.

Table 1: Overall mortality and prevalence of colibacillosis among broiler chicken.

Age group	No. of flocks screened	Total No. of birds in the flocks	Mortality		Mortality due to Colibacillosis		
			No.	%	No.	%	Proportionate distribution
0-7 days	20	29025	952	3.280	225	23.634	20.969
8-14 days	23	22064	1021	4.627	256	25.073	23.858
15-21 days	30	36007	1223	3.397	365	29.845	34.016
22-28 days	25	24500	652	2.661	168	25.767	15.657
>29 days	20	19800	223	1.126	59	26.457	5.498
Total	118	131396	4071	3.098	1073	26.357	

Prevalence of *Escherichia coli* Serogroups among Broiler Chicken

A total number of 263 bacterial isolates obtained from various organs like heart, liver, spleen, lungs, and joints were sent to National Escherichia and Salmonella, Institute, Kasauli-173204 (H.P) for sero grouping on the basis of O antigen and the observed prevalence is given in Table 2. The isolates belonged to 13 serogroups with 40 (15.21%) and 16 (6.08%) isolates classified as untypeable and as rough. Out of the 13 serotypes, the predominant isolates included O76 (15.59 %), O8 (14.45 %), and O1 (12.17 %) followed by O26 (7.22 %), O20 (6.44 %), O114 (4.94 %), O11 (4.18%), O2 (3.80 %) and O45, O84 (3.04 %) respectively.

O8 was mainly isolated from heart, O1, O76, O45, O20 were isolated from lungs and heart. Various isolates like O11 and O20 were isolated from joint fluids. O26, O84 and O1 were also isolated from spleen also. O114 was isolated from bursa. Some other isolates were classified as untypeable.

Table 2: Prevalence of *Escherchia coli serogroups* in colibacillosis affected broiler chicken.

S. No	Sero group	No. of isolates	Percentage	S. No	Sero group	No. of isolates	Percentage
1	O2	10	3.80	8	O1	32	12.17
2	045	8	3.04	9	O84	8	3.04
3	Untypeable	40	15.21	10	O8	38	14.45
4	Rough	16	6.08	11	O11	11	4.18
5	O76	41	15.59	12	O20	17	6.46
6	O114	13	4.94	13	O26	19	7.22
7	O59	10	3.80	Total		263	100

Impression Smears

Impression smears from affected organs like heart and liver stained with Wright's Giemsa and Methylene blue stain revealed presence of capsulated, cocobacillary organisms. Staining with gram's staining technique showed gram negative organisms.

Isolation and Biochemical Characterization

Escherichia coli infection was confirmed by isolation and biochemical characterization. Pink colonies on Mac Conkey agar and greenish colonies with metallic sheen on Eosin Methylene Blue agar after an overnight incubation were confirmed as *Escherichia coli*. Microscopically, the

organisms were Gram- negative, pink, short rod, arranged singly or in pairs. All the *Escherichia coli* isolates were positive for Indole and Methyl Red test while as negative for Voges- Proskauer and Citrate Utilization test.

Discussion

Avian colibacillosis caused by *Escherichia coli* is a complex syndrome characterized by multiple organ lesions. It is considered as one of the major and principal causes of mortality either as a primary or secondary pathogen [1]. In past few years, both incidence and severity of colibacillosis have rapidly increased and current scenario alarms that it is likely to grasp its hold in future and thus impose a great threat to poultry industry [2].

In the present study *E. coli* has been isolated from a total of 118 poultry farms. Out of 263 isolates only 223 isolates could be typed. Various serotypes were identified as O2, O45, O76, O114, O59, O1, O84, O8, O11, O20 and O26. Among the strains isolated O76 was most prevalent (15.59%) followed by O8 (14.45%), 026 (7.22%), O20 (6.46%), O11 (4.18%), O2 (3.8%), O45and O84 (3.04%).

Rough strain comprised 16 isolates (6.05%), and 40 isolates (15.21%) were untypeable, which could be due to use of antibiotics and presence of mixed bacterial and viral infections that could not be diagnosed clinically [3, 4]. Rough isolates were isolated mainly from joints and in some cases from heart, while rest of the strains were isolated from organs like heart, liver, lungs and spleen indicating septicaemic nature of the disease.

Prevalence of various isolates has been reported from time to time from various parts of the country [5, 6, 7] and their findings are concurrent with the findings of present study with few exceptions.

Conclusions

Colibacillosis constituted significant component of mortality among broiler chicken of all age groups ranging from 23.634% to 29.845% with overall mean of 26.357%. The most prevalent serogroup of *E. coli* observed was O76 (15.59 %), followed by 14.45 % O8, 12.17 % O1, 7.22 % O26, 6.44 % O20, 4.94 % O114, 4.18% O11, 3.80 % O2, and 3.04 % each O45 and O84. However, 15.21% untypable, and 6.08% rough type were also observed.

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