

Original Research Article

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Effect of Age and Season on Enteritis and Antibiotic Sensitivity Test of *E. coli* Isolated from Infected Chickens in Odisha, India

Mayadevi Majhi¹, Jasmine Pamia^{1*}, Susen Kumar Panda¹,
Lipismita Samal² and Rajashree Mishra³

¹Division of Veterinary Pathology, C.V.Sc. and A.H., OUAT, Bhubaneswar

²Division of Poultry Science, C.V.Sc. and A.H., OUAT, Bhubaneswar

³Division of Veterinary Microbiology, C.V.Sc. and A.H., OUAT, Bhubaneswar

College of Veterinary Science and Animal Husbandry

Orissa University of Agriculture and Technology

Bhubaneswar, India

*Corresponding author

ABSTRACT

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The incidence of enteritis in chicken was assessed in two organized farms of Odisha over a period of one and half years from January 2016 to July 2017. Detailed information about the farms like location of the farms, genotype of birds, age of birds, mortality, and cause of death were collected and noted. Blood samples from 120 numbers of enteritis affected birds and 10 numbers of apparently healthy birds of the same flock were examined for hematological parameters. The incidence of enteritis was found to be 28% from the necropsy of birds from the two organized farms. It was concluded that prevalence of enteritis in different farms was more during rainy (57%) than summer (39%) and winter (19%) seasons. The prevalence of enteritis in birds of 0-14 week's age group (51%) was more susceptible to the disease than older age groups (4%). The average values of total leucocytes count (thousands/ cubic mm) and heterophils (%) were significantly higher in the affected birds. It was revealed by etiological diagnosis of enteritis that *E. coli* has the highest involvement (80 %) than nonspecific causes (17%) and coccidiosis (3%). The antibiotic sensitivity test of avian colibacillosis showed highest sensitivity for Gentamicin.

Introduction

The basic role of poultry production is turning feed stuffs into meat. Thus healthy enteric system of growing poultry is a vital component for the success of poultry production. Any disturbance of digestive system is mostly accompanied with high

economic losses due to poor performance, increased mortality rates, decreased weight gain, increased feed conversion rates and increased medication costs. Several pathogens (bacteria, viruses and parasites) are implicated as possible cause of enteric disorders either alone or in synergy with other micro-organisms, or with non-infectious causes such

as feed and /or management related factors. In the environment of hatchery and chicken houses, the intestine of day-old chicks is immediately colonized by many species of Gram negative and positive bacteria, aerobes and anaerobes (Karpinska *et al.*, 2001). The gastrointestinal (GI) tract has the most extensive exposed surface in the body and is constantly exposed to a wide variety of potentially harmful substances. The GI tract must selectively allow the nutrients to cross the intestinal wall into the body while preventing the deleterious components of the diet from crossing the intestinal barrier (Korver, 2006). Owing to extensive scientific and technical transformation in poultry production, birds are becoming susceptible to various digestive disorders among which, enteritis is a major problem. Enteritis in chicken pose a threat to intestinal health and can contribute to poor feed efficiency, decreased weight gain with an overall increased mortality, and increased risk of contamination of poultry products for human consumption. Enteritis means inflammation of intestine. It causes increased motility of the gut and thus, decreased absorption with increased secretion. However diseases particularly enteritis acts as a major hindrance in the progress of poultry farming. Under field conditions, it is difficult to determine the true cause of enteritis. Diagnosis of enteritis can be challenging and involves a comprehensive analysis of flock history and condemnation records, clinical signs, gross and histopathology, culture and response to treatment and molecular techniques. So diagnosis of bacterial enteritis should be done at early stage in order to minimize the heavy loss to the farmers. As there is serious threat to gastrointestinal health of poultry by enteric infections, this study involves investigating colibacillosis giving emphasis to season and age of the birds so as to alleviate the risk of farmers in advance.

Materials and Methods

The present study involved two organized farms of Odisha for period of one and half years from January 2016 to July 2017. Government farms like Poultry Breeding Farm of AICRP on poultry improvement (College poultry farm) and IPDP, Laxmisagar, Bhubaneswar and others birds presented from private poultry farms to the department of Veterinary pathology, C.V.Sc and A.H were included in the study.

Epidemiological study

Retrospective data regarding the history, season, genotype of birds, total incidences, age of birds, occurrence of disease, age wise mortality rate etc. were retrieved from the farm records of Poultry Breeding Farm of AICRP on poultry improvement (College poultry farm) and IPDP, Laxmisagar, Bhubaneswar.

Hematological study

Blood samples from 120 numbers of enteritis affected birds and 10 numbers of apparently healthy birds were examined for hematological parameters like Hemoglobin, Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leucocytes Count (TLC), and Differential Count (DC). EDTA vials were used for collection of blood for hematological purpose. Blood (2-3ml) was drawn from wing vein of birds into sterile syringe and poured into the EDTA vial. Then hematological parameters were estimated. Hemoglobin concentration in the blood was determined by Sahli's acid haematin method by the help of Sahli's haemoglobinometer. TEC and TLC were counted by haemocytometer using Natt and Herrick's diluting fluid (Natt – Herrick, 1952 Technique). For differential count in chicken blood smear was prepared. Then staining was

done with a special stain known as Wright-Giemsa stain, a type of modified Giemsa stain.

Microbial examination

Impression smears from intestine and intestinal swabs were collected aseptically from fresh carcasses which died due to enteritis for Gram's staining and isolation of causative bacteria respectively.

Antibiotic sensitivity test for avian *E. coli*

Antibiotic sensitivity test for avian colibacillosis was carried out by Kirby-Bauer disc diffusion technique following CLSI (Clinical and Laboratory Standards Institute) guidelines (Wayne, 2011). The inoculum was prepared by suspending the isolate with normal saline equal to turbidity of 0.5 McFarland turbidity standards (10^6 CFU/mL). And with the help of sterile cotton swab the culture was evenly applied on the surface of the Mueller Hinton Agar (MHA) plates. Antibiotic discs (HiMedia, Mumbai) were placed on the surface of the agar using sterilized forceps. The discs were gently pressed onto the surface of the agar using flame sterilized forceps. The inoculated plates were carefully inverted and incubated for 24 hours at 37° C and the result was interpreted as per CLSI standard.

Statistical analysis

The hematological profiles of both healthy and infected birds were subjected to t-test to know the significance level of different parameters and were declared significant at $P \leq 0.05$.

Results and Discussion

The incidence of enteritis in chicken was assessed in two organized farms of Odisha over a period of one and half years from

January 2016 to July 2017. Government farms like poultry breeding farm of AICRP on poultry improvement (College poultry farm) and IPDP, Laxmisagar, Bhubaneswar and others birds presented from private poultry farms to the department of Veterinary pathology were included in the study.

Incidence of enteritis

The incidence of enteritis was found to be 28% from the necropsy of birds from the two organized farms and other private farms. A total of 1549 number of birds were presented for postmortem examination, out of which 448 numbers of birds exhibited lesions of enteritis. It was found that the birds presented from IPDP farms were more affected (68 %) than the birds received from Poultry Breeding Farm (22 %) of AICRP on poultry improvement of the college campus.

Season wise study

It was concluded that prevalence of enteritis in different farms was more during rainy (57%) than summer (39%) and winter (19%) seasons (Fig. 1). This might be due to the fact that microbial proliferation increases radically in damp and moist environment. It may be noted that the number of affected cases were more in winter (October to February) i.e., 796 and summer (March to June) i.e., 702 but mortality percentage of the birds due to enteritis was not as high as compared to rainy season (July to September) with presentation of only 51 birds but 29 of them were affected with enteritis. Rahman *et al.*, (2004) recorded colibacillosis in all three seasons of the year with significantly high rate during summer season at BRAC Poultry Disease Diagnostic Centre, Gazipur, Bangladesh. Hermans and Morgan (2007) observed high disease prevalence during the months of October to February in United Kingdom. Balasubramaniam and Dorairajan (2009) analyzed the influence of season on the

mortality pattern of poultry in Namakkal belt, Tamil Nadu and found high Incidence of chronic respiratory disease and colibacillosis with no influence of season on their occurrence. They also observed the high incidence of necrotic enteritis in winter (2.53%) and rainy season (1.84%) as compared to summer season. Datta *et al.*, (2013) studied on the epidemiology of enteritis in broiler chickens in Haryana and reported highest prevalence of enteritis in rainy and winter seasons.

Age wise variation

The prevalence of enteritis in different farms revealed that birds of 0-14 week's age group (51%) were more susceptible to the disease than older age groups (Fig. 2). This may be due to resistance of adult birds and young birds naturally susceptible to infectious conditions. Out of 448 birds affected with enteritis, 228 numbers of birds of age group 0-14 weeks were found affected with enteritis followed by 131 birds of 15-28 weeks, and rest 83 birds were more than 29 weeks of age group. Rahman *et al.*, (2004) found widely prevalence of avian colibacillosis in all age groups of chickens of Gazipur, Bangladesh with especially high prevalence rate in adult layers. Daryoush *et al.*, (2011) evaluated the epidemiological prevalence of the various kinds of enteritis in association with age, strain and sex in Azerbaijan Province of Iran. One-sample chi-square test, revealed that the highest rate of occurrence (78.57%), belonged to broilers ($p < 0.01$). It was observed greatly in 4-6 weeks of age ($p < 0.01$). Datta *et al.*, (2013) recorded maximum cases of enteritis in 29-35 days of age with 30.35% prevalence from 481 samples of broiler birds at Haryana.

Etiological study

It was revealed by etiological diagnosis of enteritis that *E. coli* has the highest involvement (80 %) than nonspecific causes

(17%) and coccidiosis (3%) (Fig. 3). From 448 enteritis cases it was found that 359 cases were positive for colibacillosis. Nonspecific causes included primarily cases of anemia, weakness, debility along with enteritis. Islam *et al.*, (2004) isolated *E. coli* from broiler birds affected with characteristic lesions of omphalitis, yolk sac infection, fibrino pericarditis, perihepatitis, hemorrhagic enteritis and accumulation of excess pericardial and peritoneal fluid. Rahman *et al* (2007) examined a total 8169 dead chickens (2960 chicks, 1083 grower and 4126 adults) from Gazipur, Bangladesh. They collected related samples like liver, spleen and intestine and then cultured on different bacteriological agar media and found salmonellosis in 53.90% of chicks (group 1) followed by omphalitis in 28.42%, colibacillosis in 13.36%, mycoplasmosis in 2.55%, necrotic enteritis in 1.18% and infectious coryza in 0.59%.

The bacterial diseases salmonellosis, colibacillosis, infectious coryza and necrotic enteritis were detected in 55.96, 11.93, 29.91 and 2.20% of group 2 (growers), respectively. Bonia *et al.*, (2010) studied on the incidence and pathological conditions in chicken of Kalinga Brown breed in Guwahati, Assam. They screened total of 120 chicken of Kalinga Brown breed for the prevalence of various disease conditions and isolated *E. coli* in all the affected cases.

Bhalerao *et al.*, (2011) studied the incidence and pathological lesions of *E. coli* infection in natural cases of poultry carcasses in Haryana. They isolated *E. coli* from blood and liver samples in 86.6% cases. Dutta *et al.*, (2011) detected and characterized Shiga toxin producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) from rectal swabs, intestinal contents, heart blood and spleen of 19 poultry birds of Aizawl, Mizoram that died due to acute diarrhea.

Hematology

The average values of TLC (thousands/ cubic mm) and heterophils (%) were significantly higher in the affected birds than apparently healthy birds (Table 1). There was no significant difference between the average hemoglobin value of the affected birds (10.40 g/dl) and apparently healthy birds (10.17 g/dl). The average TLC in the affected birds (46667 thousands/ cubic mm) showed significant increase in comparison to apparently healthy birds (20000 thousands/ cubic mm). The average values of PCV% showed no significant difference between the affected birds (36) and apparently healthy birds (38). There was no significant difference between the average TEC value of the affected birds (3.40 million per cubic mm) and apparently healthy birds (3.10 million per cubic mm). Average heterophils % of the affected birds (48.67% was found to be significantly higher than apparently healthy birds (25.00 %). The average value of the lymphocytes % of affected birds (47.00)

showed significantly lower value in comparison to the values (69.00) of apparently healthy birds. The average value of monocytes % of affected birds (2.0) showed significantly lower value in comparison to the values (8.33) of apparently healthy birds. Average value of the eosinophils % (1.67) and basophils % (1.33) showed a lower value than the corresponding values (2.00 and 1.00) of the apparently healthy birds. Arukha (2015) found that the average values of haemoglobin, TLC and DC were significantly higher in the affected birds than apparently healthy birds of the same flock. Average heterophils % of the affected birds (67.65%) was found to be significantly higher than apparently healthy birds (35.57%). Mishra (2014) revealed that the hemoglobin, TEC, PCV and heterophils percentage were increased due to heat stress in chicken. Guabiraba and Schouler (2015) observed that heterophils would arrive very quickly at the infected sites probably due to a robust production of CXC chemokines during bacterial infection.

Table.1 Comparative hematological profile of enteritis affected birds with healthy birds

Attributes	Enteritis affected birds	Healthy birds	T value	P value
Hb (g/dl)	10.40±0.64	10.17±0.73	0.241	0.822
PCV (%)	36.33±0.88	38.00±1.15	-1.147	0.315
TEC (millions/ cubic mm)	3.40±0.12	3.10±0.06	2.324	0.081
TLC (thousands/ cubic mm)	46667 ^a ±2186	20000 ^b ±577	11.795	0.000
Heterophils (%)	48.67 ^a ±3.48	25.00 ^b ±1.73	6.088	0.004
Lymphocytes%	47.00 ^b ±2.65	69.00 ^a ±3.21	-5.284	0.006
Monocytes %	2.00 ^b ±0.00	8.33 ^a ±2.03	-3.124	0.035
Eosinophil %	1.67±0.33	2.00±0.58	-0.500	0.643
Basophil %	1.33±0.33	1.00±0.29	0.756	0.492

^{a,b} Means bearing different superscripts in a row differ significantly

Table.2 Antibiotic sensitivity profile of chicken colibacillosis

Antibiotic	Total no. of samples	No. of susceptible samples	Sensitivity %
Gentamicin	30	27	90
Amikacin	30	24	80
Colistin	30	21	70
Cefotaxime	30	18	60
Enrofloxacin	30	15	50
Ciprofloxacin	30	12	40
Ampicillin	30	6	20
Chloramphenicol	30	6	20
Lincomycin	30	3	10

Fig.1 Season wise mortality

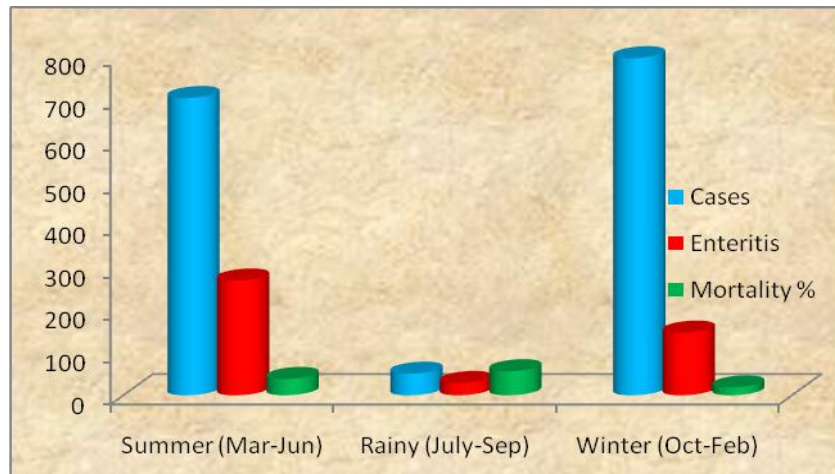


Fig.2 Age wise mortality



Fig.3 Etiological classification of enteritis

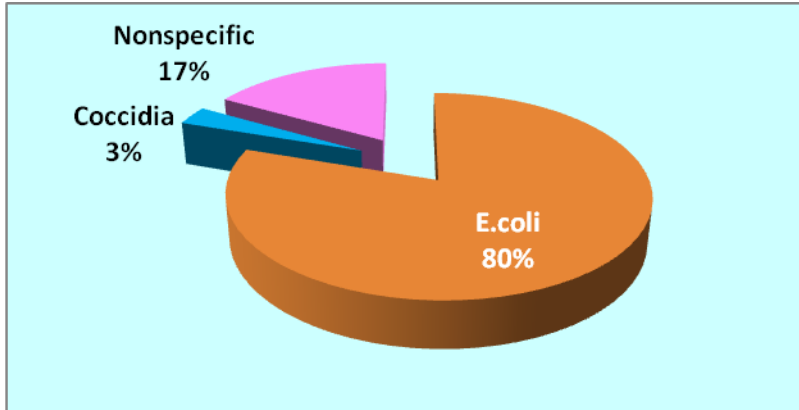
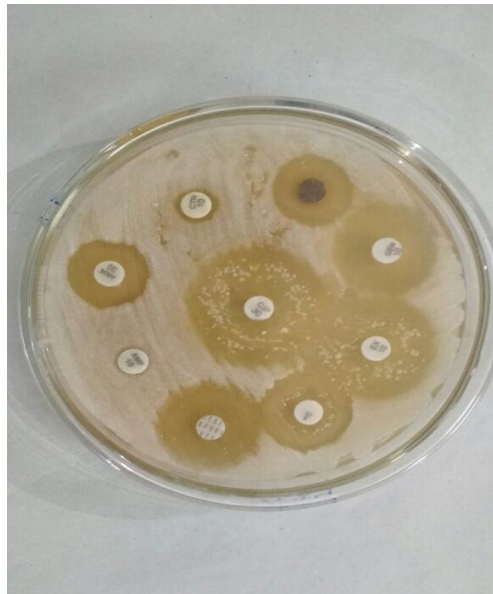


Fig.4 Antibiotic sensitivity test using discs



Microbiological studies

On microscopic examination of the Gram stained impression smears of intestinal luminal surface revealed presence of large number of Gram negative (pink colour) small rods arranged in single, pair or in short chains. *E. coli* were isolated from the intestinal swabs by routine procedure on eosin Methylene Blue agar with a metallic sheen over the surface of the growth media so also on MacConkey's Lactose Agar there was

presence of pink colour colonies. Zinnah *et al.*, (2007) studied and observed all the *E. coli* isolates which produced bright pink colonies on MacConkey agar, yellowish green colonies surrounded by an intense yellow green zone on Brilliant Green agar and characteristic metallic sheen colonies on the Eosin Methylene Blue agar. In Gram's staining technique, all the isolates were pink colored, small rod shaped Gram negative bacilli. Roshid *et al.*, (2013) detected and characterized *E. coli* from gut, liver and lungs

of day old chickens. They observed black colour colony with metallic sheen in EMB media, bright pink colored smooth colonies in MC agar and pink colored smooth colonies in SS agar as a cultural characteristics arranged in single or chain as morphological characteristics. Tonu *et al.*, (2011) examined all 10 isolates after Gram's staining which revealed Gram-negative, pink coloured, short rod shaped organisms arranged as single or in pair. The morphology of the isolated bacteria from 10 isolates exhibited Gram negative, pink color, short rod to coccoid shape. Chotiah and Damayanti (2017) isolated 76.02% *E. coli* from a total of 196 samples collected from intestine, liver, heart, egg yolk, air sac and faeces.

Antibiotic sensitivity test

The antibiotic sensitivity test of avian colibacillosis showed highest sensitivity for Gentamicin (90%) followed by Amikacin (80%), Colistin (70%), Cefotaxime (60%), Enrofloxacin (50%), Ciprofloxacin (40%), Ampicillin (20%), Chloramphenicol (20%) and Lyncomycin (10%) respectively (Table 2 and Fig. 4). Saidi *et al* (2016) showed that there were high prevalence of sensitivity of avian pathogenic *E. coli* (APEC) to ciprofloxacin (100%) and gentamycin (97.1%). The isolates showed moderate rates of sensitivity to chloramphenicol and neomycin in Harare, Zimbabwe. Chotiah and Damayanti (2017) isolated *E. coli* from septicaemia, sick and dead chickens collected from three districts in West Java District showing *E.coli*s sensitive to chloramphenicol and resistant to ampicillin, neomycin and sulfamethoxazole, trimethoprim. Antibiogram profiles indicated that gentamycin sensitivity was 64.5%.

It was concluded that prevalence of enteritis in different farms was more during rainy than summer and winter seasons. The prevalence

of enteritis in different farms revealed that birds of 0-14 week's age group (51%) were more susceptible to the disease than older age groups. It was revealed by etiological diagnosis of enteritis that *E. coli* has the highest involvement (80 %) than nonspecific causes (17%) and coccidiosis (3%). The average values of heterophils %, and TLC values were higher whereas average value of lymphocyte and monocytes, were significantly decreased in the affected birds in comparison to that of apparently healthy birds. The antibiotic which showed highest sensitivity for avian colibacillosis was Gentamicin.

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