

Original Research Article

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## Serotyping and Prevalence of *Escherichia coli* Infection in Poultry of South Gujarat based on Culture Isolation and Identification

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### ABSTRACT

In the present study, total 290 swab samples collected from organized poultry farms located in Navsari and Valsad districts and screened for prevalence study and different serotype of *E. coli* infection using culture isolation and identification. Isolation and identification resulted out of 290 swab samples 207 isolates produced lactose fermenting pink colored colonies on MacConkey agar, produced colonies with greenish metallic sheen on EMB agar and grams staining revealed typical gram-negative rods morphologically similar to *E. coli*. With culture isolation and identification overall prevalence was 71.38 per cent. District wise prevalence in Navsari and Valsad districts was 74.00 per cent and 65.56 per cent, respectively. Broiler birds showed higher prevalence (74.00, 148/200) rate in comparison to Layer birds (65.56, 59/90). Significantly prevalence was comparatively higher in January (90.21%) followed by September (78.78%), December (69.38%), August (51.85%) and November (40%) months. The age wise high prevalence was 82.14 per cent and 55.00 per cent in 0-30 days and 31-35 days of age group, respectively in broiler birds and 68.51 per cent and 61.11 per cent in 40-45 days and 46-50 days of age group, respectively in layer birds. As per result National *Salmonella* and *E. coli* Center, Kasauli, 15 different serotypes out of 35 isolates, two of O8, two of O35, four of O83, three of O88, two of O119 and two of O149. High prevalence of serotype O83 (11.42%) followed by O88 (8.57), O8 (5.71), O35 (5.71), O119 (5.71) and O149 (5.71%).

#### Keywords

*E. coli*, Poultry, Isolation and Identification, Serotyping, Prevalence

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### Introduction

*Escherichia coli* (*E.coli*) is a part of normal intestinal microflora of fowls and mammals. Under some conditions bacteria of the normal flora may become harmful to the host. Moreover such as a high stocking density,

poor hygiene condition, poor litter, high ammonia level and other stressful situation may reduce the resistance of the birds and increase the susceptibility to infections. *E. coli* belongs to the Enterobacteriaceae family and in India first reported of colibacillosis in brooder chicks by Gurumoorthy and

Panduranga Rao (1962). Cellulitis could develop at any age of birds and the lesions associated with other manifestation of collibacillosis like air sacculitis, pericarditis and perihepatitis in chicken mostly four to six weeks of age reported by Jonhson *et al.*, (2001).

Serotype of *E.coli* are present approximately 180 O, 60 H, and 80 K antigens. In most serological typing scheme only the O and H antigens are determined i.e. O157:H7. The O antigen determines serogroups and heat stable somatic antigen while the H antigen determines serotype and heat labile flagellar antigen.

In India poultry industry is one of the fastest growing agricultural sectors in the country. Commercial broilers are grown throughout the world and broiler chicken becoming the most affordable, delicious and nutritious protein (Kumari, 2016). The consumption of meat is increasing in India and agriculture is considered as the backbone of a majority of people. The high demand of chicken meat consumption is due to the versatility of the meat, relatively low cost in comparison to the other meat, and the acceptance of the chicken meat to all religions (Kim, 2014). Hence present experiment has been carried out to known status of *E.coli* in two districts of South Gujarat.

### **Materials and Methods**

A total 290 swab samples collected from organized poultry farms located in Navsari and Valsad districts screened for prevalence study and different serotype of *E.coli* infection using culture isolation and identification.

Media and chemicals required for isolation and biochemical characterization of *E.coli* were prepared as per Cruickshank *et al.*,

(1973) and Agrawal *et al.*, (2003). Primary isolation of *E.coli* was done using Mac Conkey agar medium. Lactose fermented pinkish colour colony selected and subcultured on Eosin Methylene Blue agar medium to detect the production of metallic sheen. All the isolates were stained by gram's staining and observed under oil immersion objective for presence of gram negative rods. Biochemical tests like Indole, Methyl-red, Voges-proskauer and Citrate utilization were employed for identification of *E.coli* (Cruickshank *et al.*, 1973; Agrawal *et al.*, 2003).

Out of 90 *E.coli* isolates, 35 isolates were selected for serotyping and sent to National Salmonella and Escherichia coli center (NSEC), Central Research Institute (CRI), Kasauli on nutrient agar stab for serotyping.

### **Results and Discussion**

The district-wise, month-wise, bird type-wise and age-wise prevalence details are given in table-1. Out of 290 swab samples tested, 207 were found positive for *E.coli* infection yielding an overall prevalence of 71.38 per cent in the birds of the Navsari and Valsad districts. District wise prevalence in Navsari and Valsad districts was 74.00 per cent and 65.56 per cent, respectively.

In the present study, broiler birds showed higher prevalence (74.00, 148/200) rate in comparison to Layer birds (65.56, 59/90). In present study higher prevalence of collibacillosis was found in broiler birds than layer which supported by earlier reports of Omer *et al.*, (2010), Srinivasan *et al.*, (2014), Matin *et al.*, (2017) and Rahman *et al.*, (2017). However, Rahman *et al.*, (2004) reported high prevalence of *E.coli* infection in layer birds. Higher prevalence in broiler than layer in present study might be due to water management and hygiene of deep litter

housing system which makes broiler more prone to infection. Good housing, hygiene and avoiding overcrowding are very important in reducing infection rates of *E.coli* (Saidi *et al.*, 2012).

Further, the prevalence of *E.coli* was significantly higher in January (90.21%) followed by September (78.78%), December (69.38%), August (51.85%) and November (40%) month (Table 4.1). Similarly, Sultana

*et al.*, (2012), Mukhtar *et al.*, (2012) and El Sayed *et al.*, (2015) also reported higher prevalence of colibacillosis in winter months. This variation may be attributed to variation in the environmental and hygienic condition in poultry farms. Overcrowding, poor ventilation and high amount of ammonia in air may be the attributing factors to increased incidence of *E.coli* in water, feed, litter and air in winter than summer (Mohamed *et al.*, 2014).

**Table.1** District, months, bird type wise and age wise prevalence of *E.coli* infection in birds

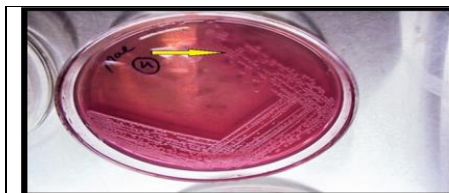
Particular	No. of sample (n= 40 broiler and n=18 layer)	No. of Positive sample	Percentage of positive sample	
<b>1. District</b>				
Navsari	200	148	74.00	
Valsad	90	59	65.56	
<b>Total</b>	290	207	71.38	
$X^2$	2.17 (p=0.14)			
<b>2. Months</b>				
August	27	14	51.85	
September	33	26	78.78	
November	40	16	40.00	
December	98	68	69.38	
January	92	83	90.21	
<b>Total</b>	290	207	71.38	
$X^2$	41.38** (p=0.00)			
<b>3. Bird type</b>				
Broiler	200	148	74.00	
Layer	90	59	65.56	
<b>Total</b>	290	207	71.38	
$X^2$	2.17 (p=0.14)			
<b>4. Age</b>				
<b>Broiler</b>	0-30 days	140	115	82.14
	31-35 days	60	33	55.00
<b>Layer</b>	40-45 days	54	37	68.51
	46-50 days	36	22	61.11
<b>Total</b>	290	207	71.38	
$X^2$	17.90** (p=0.00)			
<b>Note: ** Highly Significant at P &lt; 0.01</b>				

**Table.2** Percent Positive samples of *E.coli* in Layer and Broiler birds with different sample collection site

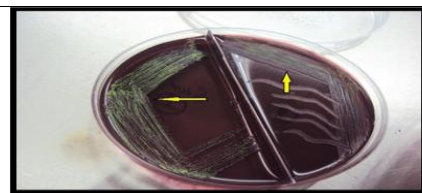
Total Layer (n=18)	Sample site with sample number	Layer (Positive samples)	%	Total Broiler (n=40)	Sample site with sample number	Broiler (Positive samples)	%
18	Liver (18)	14	77.78	40	Liver (40)	35	87.50
	Heart (18)	13	72.22		Heart (40)	34	85.00
	Abdominal fluid (18)	13	72.22		Abdominal Fluid (40)	31	77.50
	Yolk sac (18)	3	16.67		Spleen (40)	12	30.00
	Intestine (18)	16	88.89		Intestine (40)	36	90.00
<b>Total</b>	<b>90</b>	<b>59</b>	<b>65.56</b>	<b>Total</b>	<b>200</b>	<b>148</b>	<b>74.00</b>

**Table.3** Farm wise prevalence of *E.coli* infected Poultry

Sr. No.	Location	Number of birds	Number of samples	Number of Positive samples	Percentage of positive samples
1.	Village:Gourgaon, Valsad	18	90	59	65.55
2.	Village: Abrama, Navsari	12	60	54	60.00
3.	Village:Eru gam, Navsari	11	55	42	76.36
4.	Village: Machad, Navsari	04	20	12	60.00
5.	Village: Mandir, Navsari	07	35	17	48.57
6.	Village: Chikhli, Navsari	06	30	23	76.66
	<b>Total</b>	<b>58</b>	<b>290</b>	<b>207</b>	<b>71.38</b>



**Figure:** *E.coli* isolates showing rose pink colored colonies on Mac Conkey agar



**Figure:** Isolation of *E.coli* showing characteristics greenish colored colonies with metallic sheen on EMB agar



**Figure:** IMViC biochemical test for *E.coli*. Where A indicate Indole test, B indicate Methyl red, C indicate Voges-Proskauer test and D indicate Citrate utilization test.

From the above mentioned results of table-3, it is obvious that *E.coli* isolates were recovered from poultry farms with higher prevalence from intestine sample followed by liver and lung which was also nearly similar result obtained by Yousef *et al.*, (2013).

The prevalence of colibacillosis was differ significantly between different age group of broiler and layer birds. Where in significantly highest 82.14 per cent prevalence of colibacillosis was seen in 0-30 days age group followed by 55.00 per cent prevalence in 31-35 days age group of broiler birds. While in layer, 68.51 per cent prevalence was seen in 40-45 days age group followed by 61.11 per cent prevalence in 46-50 days age group (Table 4.1).

Biochemical characterization of 207 *E.coli* isolates was done with Indole test, MR test, VP test, Citrate utilization test and Motility test. Resulted 207 isolates 90 isolates confirmed as *E.coli* with Indole positive, MR test positive, VP test negative, Citrate negative and also all isolates were found motile which were similar with the findings of Buxton and Fraser (1977). No atypical or unusual biochemical reaction was shown by any of the 90 isolates tested.

In present study obtained 15 serotypes out of 35 isolates, two of O8 (5.71), two of O35 (5.71), four of O83 (11.42), three of O88 (8.57), two of O119 (5.71) and two of O149 (5.71). High prevalence of O83 serotype which also further confirmed by PCR using *stx1* and *stx2* gene sequence primer. The fact that O83 has been associated with human illness (Bettelheim, 2007) raised the possibility that O83 might be transferred to humans via aerosol route supported by Xia *et al.*, (2010).

The isolated serotype O8 in present study was already confirmed as virulent serotype by

Kika *et al.*, (2013). Further these serogroups are commonly associated with avian colibacillosis and confirm their role as potential pathogens in the extra intestinal infections of poultry (Dho-Moulin and Fairbrother, 1999; Ewers *et al.*, 2004). Occurrence of different *E. coli* serotypes which were involved in coli septicaemia and other conditions in chicken were reported from time to time by various researchers *viz.*, Saikia *et al.*, (1995) and Rama Devi (2005). They opined that the outbreaks of the disease might be influenced by the various strains present in the environment and to the extent of intensity of the strains present.

The serotyping of O groups is in corroboration with the findings of Gross (1994), Saikia *et al.*, (1995) and Rama Devi (2005). It was observed by the researchers that the serotypes of O were commonly encountered in various respiratory, septicemic and colibacillosis condition in chicken and they identified as many as 154 O serotypes. These O serotypes were prevalent worldwide with number of serotypes identified as cause of poultry disease (Gross, 1961).

In present study recording of serotype O149 was in accordance with the findings of Frydendahl (2002) where in the researcher reported these one serotype in piglet diarrhoea. It is obvious to say that in multifarming system there is every possibility of cross contamination of serotypes of one species to another. So in the present study samples were collected from the poultry sources which were located nearer to free ranging of pig and cattle population. Therefore it could be possible for cross contamination of *E. coli* serotype from one species to another by possible environmental influences and which could be trespassing of the workers similar result reported by Blanco *et al.*, (1997).

Serotype O88 isolates by Srinivasan *et al.*, (2014) from commercial caged layer chickens was supported result of present study. Knobl *et al.*, (2011) reported serotype O88 as an Avian Pathogenic *Escherichia coli* (APEC). O119 serotype was isolated in present study. Similar O119 serogroup was also isolated by H.Kh *et al.*, (2014) and confirmed as Enteropathogenic *E.coli* (EPEC). Serotype O35 reported by Cloud *et al.*, (1985) from broiler birds affected with colibacillosis except from yolk sac infection sample which was similar to present study.

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