

Original Research Article

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Influence of Age, Sex and Season on the Occurrence of *Escherichia coli* O157: H7 in Sheep and Goats of Hyderabad-Karnataka Region, India

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ABSTRACT

E. coli O157:H7 is an emerging food borne pathogen having zoonotic importance. Though the primary reservoir of this serotype is cattle; Sheep and goats are also considered to be the main reservoir for *E. coli* O157:H7. In the present study, faecal samples were collected from sheep (n=517) and goats (n=450) in different farms across Hyderabad-Karnataka region during June 2016 to August 2017 were analysed. The samples were processed and analysed for the isolation, identification and confirmation of *E. coli* O157:H7 by cultural, biochemical characterization and latex agglutination test. The prevalence is more in male (4.14 % in sheep and 2.94 % in goats) than female (3.26 % in sheep and 2.84 % in goats), also in yearlings (6.25 % in sheep and 5.08 % in goats) than lambs or kids (3.35 % in sheep and 4.22 % in goats) and adult sheep or goats (2.02 % in sheep and 1.56 % in goats). The prevalence of *E. coli* O157:H7 varied between seasons with the highest prevalence occurring in summer (3.70 % in sheep and 3.70 % in goats) followed by monsoon (3.30 % in sheep and 1.81 % in goats) and winter seasons (2.79 % in sheep and 2.30 % in goats). Understanding above factors will contribute to shedding of *E. coli* O157:H7 in small ruminants which help in development of control strategies to reduce pre-harvest incidence of food borne pathogen in humans.

Keywords

E. coli O157: H7, sheep and goats, latex agglutination test, Hyderabad-Karnataka region, age, sex and season

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Introduction

Escherichia coli are genetically heterogeneous group of bacteria whose members are typically non-pathogenic that form a part of the normal intestinal microflora of humans and animals. However, certain subsets of this bacterial species have acquired genes that enable them to cause intestinal or extra intestinal disease. One of these pathotypes, called Shiga toxin-producing *E. coli* (STEC), refers to those strains of *E. coli* that produce at least one

member of a class of potent cytotoxin called Shiga toxin (Stx). A subgroup of STEC causes watery diarrhoea in humans which can progress to haemorrhagic colitis and potential systemic complications due to the action of Shiga toxin (Kaper *et al.*, 2004). These are referred as Enterohaemorrhagic *E. coli* (EHEC).

About 200 EHEC serotypes have been isolated from animal and food sources (Denis *et al.*, 2012). The most significant EHEC of humans

is *E. coli* O157:H7. After the first outbreak of bloody diarrhoea in Oregon and Michigan, U.S.A. in 1982, *E. coli* O157:H7 has become the most widely known EHEC strain (Riley *et al.*, 1983, Wells *et al.*, 1983). Because of the severity of these illnesses and the apparent low infective dose (< 10 cells), *E. coli* O157:H7 is considered one of the most serious known food borne pathogens.

Important reservoirs of pathogenic *E. coli* O157:H7 in the environment have been ruminants, particularly cattle, sheep and goats which are asymptomatic carriers (Al-Saigh *et al.*, 2004). These asymptomatic carriers normally shed the organism in faeces contaminating soil and surface waters (Hilborn *et al.*, 1999). Transmission to people occurs primarily via ingestion of inadequately processed contaminated food or water and less frequently through contact with manure, animals, or infected people.

While a number of factors have been identified and suggested as playing a role in the on-farm population dynamics of this pathogen, there are very few reports and very few researchers have worked on ecology and epidemiology of shedding pattern of *E. coli* O157:H7 in small ruminants compared with the number of surveys done in cattle especially with respect to age, sex and seasonal variation.

Because of its public health importance, *E. coli* O157:H7 impacts production security, trade and consumer confidence for meat and chevon and meat derived foods in a significant way. The pathogen reduction efforts applied throughout the animal production and processing chain should reduce the risk of *E. coli* O157:H7 occurrence in the final meat products. A better understanding of the epidemiology and shedding pattern of *E. coli* O157:H7 could offer possible strategies to reduce the carriage and shedding of the

organism by sheep and goats that in turn could reduce the risk of human infection.

Keeping in view with the above facts, the present research work was under taken to isolate and confirm *E. coli* O157:H7 from faecal samples of sheep and goats by cultural identification, biochemical characterization and latex agglutination test. And also to study shedding pattern of *E. coli* O157:H7 in different age group, sex and seasons from faecal samples collected from sheep and goats in different farms across Hyderabad-Karnataka region.

Materials and Methods

Collection of samples

In the present study, faecal samples collected from sheep (n=517) and goats (n=450) of different age groups (lambs or kids, yearlings and adult), sex and in different seasons from different farms across Hyderabad-Karnataka region during June 2016 to August 2017 were analysed.

The faecal samples were collected in a sterile vial directly from rectum by sterile cotton swab stick (Hi Media, Mumbai, India) and also as faecal pellet directly collected in sterile test tube. They were labelled properly and transported to laboratory in thermo-cool container jacketed with ice packs. The samples were processed and analyzed for the isolation of *E. coli* O157:H7 within 24 hours.

Cultural isolation of *E. coli* O157:H7 and biochemical characterization of isolates

One grams of each faecal sample was inoculated into 9 ml (1:9 ratio) modified tryptone soya broth (mTSB) supplemented with novobiocin and incubated overnight at 37°C for 24 hours. A loopful of inoculum from the enrichment medium was inoculated

onto selective isolation media Sorbitol-MacConkey agar supplemented with cefixime (0.025 mg) and potassium tellurite (1.25 mg) (CT-SMAC). The agar plate was incubated for 24 hrs at 37°C. Growth of typical colourless colonies (sorbitol negative) indicate positive for *E. coli* O157:H7.

These isolates were further subjected for isolation on Eosin Methylene Blue (EMB) agar, to confirm the isolate as *E. coli* by observing the colonies producing metallic sheen. The sorbitol negative colourless colonies were taken for further confirmation by Gram's staining and biochemical tests like IMViC (Indole, Methyl Red, Vogues Proskeur and Citrate utilization) tests, glucuronidase, nitrate reduction, lysine utilization, ONPG (Orthonitrophenyl galactosidase) and sugar fermentation tests (lactose, glucose, sucrose, and sorbitol) using KB010 *E. coli* identification kit (Hi Media, Mumbai, India).

The typical colourless isolates on CT-SMAC agar showing metallic sheen colonies on EMB agar, gram negative staining character, positive for indole, methyl red, glucuronidase, nitrate reduction, lysine utilization, ONPG and sugar fermentation (lactose, glucose, sucrose) and negative for Vogues- Proskauer test, Citrate utilization and sorbitol fermentation were presumed as positive for *E. coli* O157:H7 and inoculated into mTSB for preservation. For the confirmation of the samples found positive for *E. coli* O157:H7 by cultural isolation and biochemical tests the isolated colonies on CT-SMAC agar were tested for the presence of O157 and H7 antigens by latex agglutination using Wellcolex® *E. coli* O157:H7 kit.

Results and Discussion

The present study was undertaken for the identification of *E. coli* O157:H7 from faecal samples of sheep and goats by cultural

identification, cultural isolation with biochemical characterization and latex agglutination test and to find the variation in epidemiology of shedding pattern of *E. coli* O157:H7 in small ruminants, between different age groups, sex and among seasons.

The results of cultural isolation, biochemical characterization and Latex agglutination test of *E. coli* O157:H7 in sheep and goats from faecal samples is presented in the Fig 1 and 2.

The confirmed diagnosis of sorbitol negative *E. coli* O157:H7 was performed by KB 010 Hi *E. Coli* identification kit for biochemical tests and the results were obtained depending on interpretation kit chart and result entry was done in datasheet. All isolates showed positive result as shown in the Fig. 3. Further the samples found positive for *E. coli* O157:H7 by cultural isolation with biochemical characterization were subjected for confirmative identification by observing the presence of the O157 and the H7 antigens using latex agglutination test. The isolates positive for O157 and the H7 antigens showed agglutination reaction, respectively, as shown in the Fig IV.

In this study, out of 517 sheep faecal samples analyzed, 241 samples were from males and 276 samples were from female. Based on the latex agglutination test for identification of *E. coli* O157:H7, the percent of female animals that showed shedding of *E. coli* O157:H7 was only 3.26 % (only 9 out of 276 samples), whereas, the percent of male that showed shedding was 4.14 % (10 out of 241 samples).

In goats out of 450 faecal samples analyzed, 204 samples were from males and 246 samples were from females. Based on the latex agglutination test for identification of *E. coli* O157:H7, the percent of female animals that showed shedding of *E. coli* O157:H7 was only 2.84 % (only 7 out of 246 samples),

whereas, the percent of male that showed shedding was 2.94 % (6 out of 204 samples).

Age wise variation in shedding pattern of *E. coli* O157:H7 was analysed between lambs or kids, yearlings and adult. Based on the latex agglutination test of *E. coli* O157:H7, the variation in shedding pattern of *E. coli* O157:H7 in faeces of sheep and goats shown in table-1. The results indicate that age influences the shedding of *E. coli* O157:H7 in faeces and the shedding was more in lambs and yearlings than in adult sheep and goats.

Seasonal variation in shedding pattern of *E. coli* O157:H7 was analysed between three seasons, viz; summer, monsoon and winter. Out of 517 sheep faecal samples were analysed from June 2016 to August 2017, 162 faecal samples were collected in summer season, 212 faecal samples were collected in monsoon season and 143 faecal samples were collected in winter season.

Based on the latex agglutination test identification of *E. coli* O157:H7, the percent of sheep that showed shedding of *E. coli* O157:H7 in faeces in winter, mansoon and

summer season respectively was 2.79% (4 out of 143 samples), 3.30 % (7 out of 212 samples) and 3.70 % (6 out of 162 samples).

Out of 450 goats faecal samples collected and analysed from June 2016 to August 2017, 135 faecal samples were collected in summer season, 165 faecal samples were collected in monsoon season and 130 faecal samples were collected in winter season. Based on the latex agglutination test identification of *E. coli* O157:H7, the percent of goats that showed shedding *E. coli* O157:H7 in faeces in winter, mansoon and summer seasons respectively as 2.30 % (3 out of 130 samples), 1.81 % (3 out of 165 samples) and 3.70 % (5 out of 135 samples). The results indicated that season influences the shedding of *E. coli* O157:H7 in faeces.

Further seasons wise monthly trend of shedding pattern were also analysed. It was observed that maximum percentage of faecal samples positive for *E. coli* O157:H7 in sheep and goats were found in the month of April followed by decreased shedding pattern with least percentage of samples positive for *E. coli* O157:H7 in the month of December.

Fig.1 Prevalence of *E. coli* O157:H7 in the faecal samples of sheep by using different methods

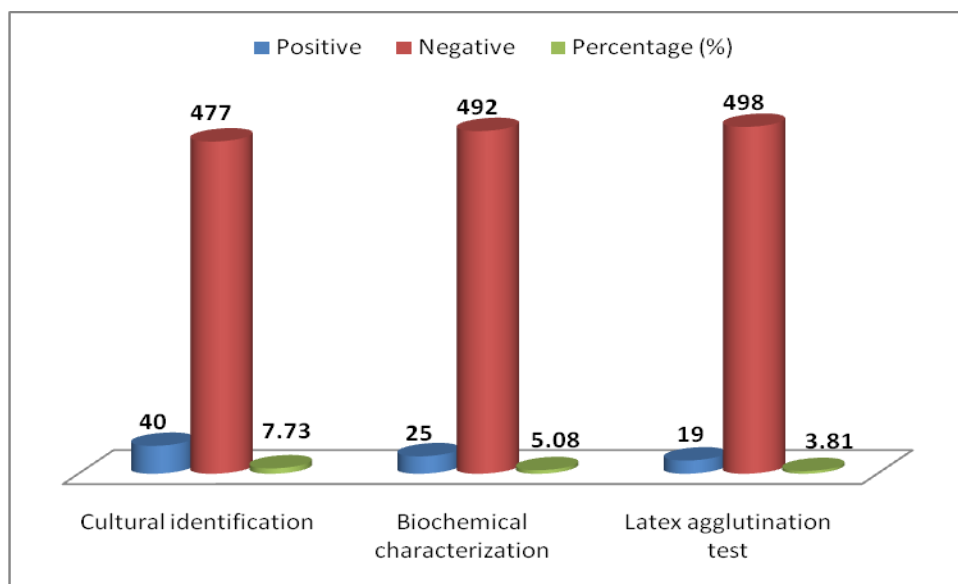


Fig.2 Prevalence of *E. coli* O157:H7 in the faecal samples of goats by using different methods

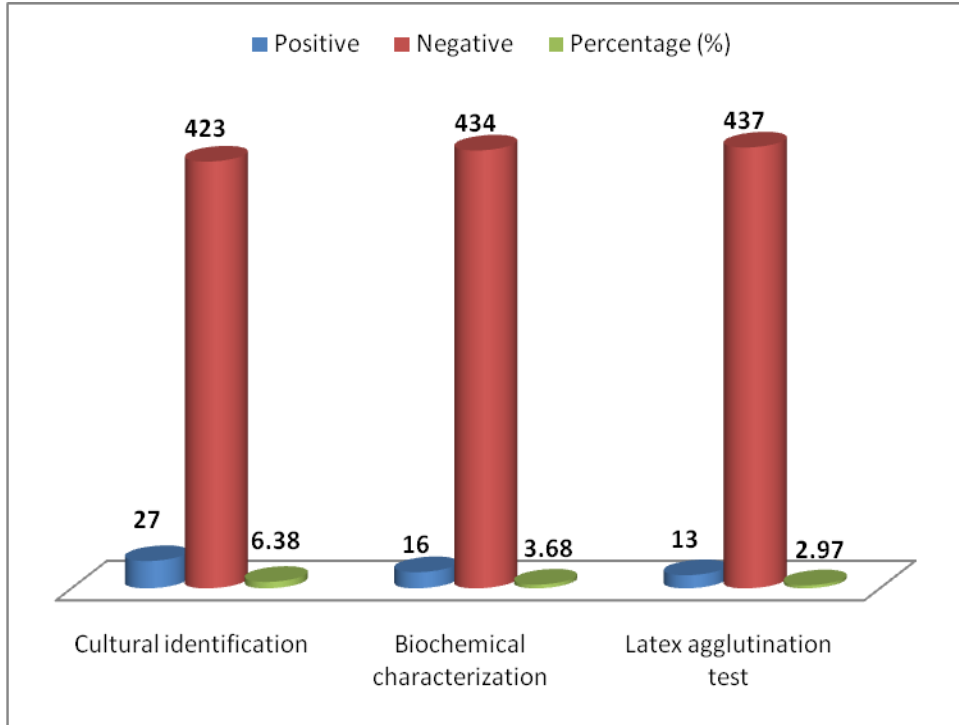


Fig.3 Sample positive for *E. coli* O157:H7 showing biochemical characterization



- | | | | |
|------------------|--------------------------|------------------------|-----------------------|
| 1. Methyl Red | 2. Voges- Proskauer test | 3. Citrate utilization | 4. Indole |
| 5. Glucuronidase | 6. Nitrate reduction | 7. ONPG | 8. Lysine utilization |
| 9. Lactose | 10. Glucose | 11. Sucrose | 12. Sorbitol |

Fig.4 Sample positive for O157 and the H7 antigens showing agglutination reaction in 2st and 6th circle similar to positive controls, indicating positive for *E. coli* O157:H7

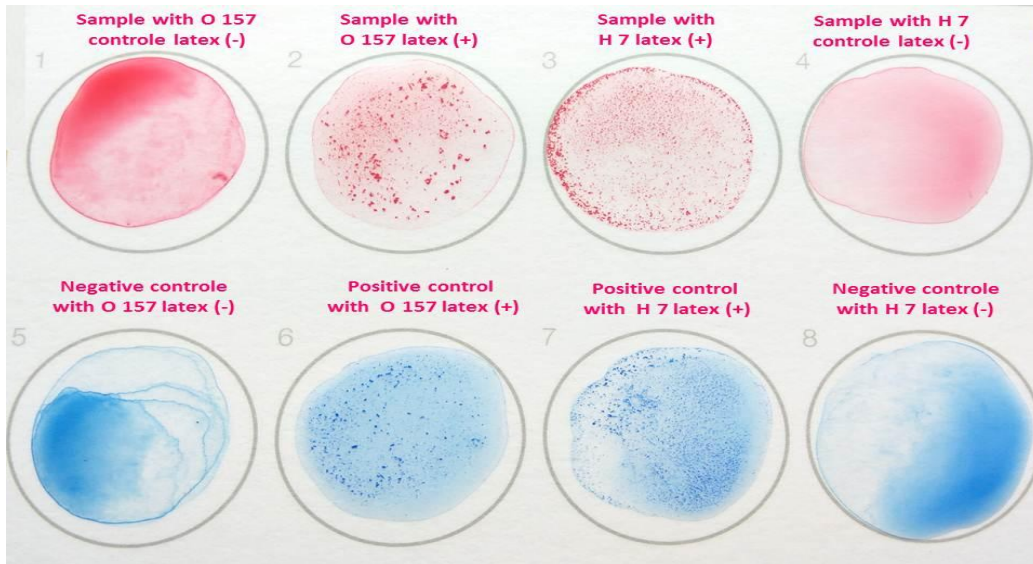


Table.1 Age wise variation in the shedding of *E. coli* O157:H7 in the faecal samples of sheep and goats

Category of animal	Sheep			Goat		
	Lambs	Yearlings	Adult	Kids	Yearlings	Adult
Positive	5	6	6	3	3	5
Negative	144	90	266	68	56	314
Total	149	96	272	71	59	319
Percentage (%)	3.35	6.25	2.20	4.22	5.08	1.56

In the present study, *E. coli* O157:H7 from faecal samples was isolated by enriching in mTSB. mTSB as enrichment medium specifically for *E. coli* O157:H7 has been recommended by ISO committee under specification, ISO/DIS16654:1999 (ISO, 2015). *E. coli* O157:H7 rapidly ferments lactose and is indistinguishable from most other *E. coli* on traditional lactose containing media. However, *E. coli* O157:H7 cannot ferment sorbitol within 24 hrs, while 90% of *E. coli* can. This character was used as a criterion for differentiating it from other *E. coli* (Ahmad *et al.*, 2013; Faten and Afaf, 2013; Adamu *et al.*, 2014). Centers for Disease Control and Prevention had also recommend SMAC as selective indicator

media for *E. coli* O157:H7 (CDC, 2009). In addition, Orth *et al.*, (2007) showed the resistance of *E. coli* O157:H7 for tellurite and therefore grows in concentration which often inhibits most other *E. coli*. Therefore, CT-SMAC was used as selective and differential medium in this research work. Only the typical sorbitol negative colonies were considered as positive for *E. coli* O157:H7. As such there is no difference in the biochemical characters between *E. coli* O157:H7 (Visetsripong *et al.*, 2007) and other serotypes of *E. coli* except sorbitol fermentation, i.e., *E. coli* O157:H7 being sorbitol negative and other serotypes being sorbitol positive (Bettelheim, 2007; Adamu *et al.*, 2014).

The isolates positive for *E. coli* O157 and the H7 antigens showed agglutination with latex coated with respective monoclonal antibodies in latex agglutination test. Since monoclonal antibodies react very specifically with their respective antigens latex agglutination test was used for the confirmation of the isolates as *E. coli* O157:H7. USDA FSIS recommends use of latex agglutination test for the confirmation of *E. coli* O157:H7 from meat products and environmental samples. In this study, the latex agglutination test, which is being very sensitive and specific for the two specific antigens of *E. coli* O157:H7 was taken as gold standard test to compare and estimate epidemiological sensitivity and specificity of cultural isolation method, cultural isolation with biochemical identification.

The results indicated that sex of animals influenced the shedding of *E. coli* O157:H7 in faeces. Hormonal differences in males and female animals could be the reason for higher shedding of this pathogen in male compared to females as it was concluded by earlier workers (Schultz *et al.*, 2005; Schroeder and Sasha, 2005).

The results indicated that age influenced the shedding of *E. coli* O157:H7 in faeces and the shedding was more in lambs and yearlings than in adult sheep and goats. This may be due to reason that young weaned yearlings were found to shed *E. coli* O157:H7 at higher levels and for longer period than adult sheep and goats, which could be due to dietary stress during weaning, and because they do not yet have a fully formed rumen to help suppress *E. coli* O157:H7 growth (Hussain and Bolinger, 2005). Low prevalence of *E. coli* O157:H7 in lambs or kids may be related to the presence of protective antibodies in colostrum and milk, as indicated by results of some studies with colostrum deprived animals. The findings in the present study and

the above references support the generalisation of presence of an age-dependent variation in prevalence of *E. coli* O157:H7 (Riley *et al.*, 2003).

Seasonal variation in *E. coli* O157:H7 carriage and transient shedding patterns in sheep have been demonstrated, with peak prevalence in summer months. The result in the present study indicated that prevalence of shedding of this pathogen typically increases during the summer months and is lowest in the winter which is same as was concluded by earlier workers (Chapman *et al.*, 1997; Hancock *et al.*, 2001; Edrington *et al.*, 2004; Hussein *et al.*, 2005; Edrington *et al.*, 2006; Gautam *et al.*, 2011). The reason for seasonal variation may be because many mammalian species undergo seasonal changes in immune system function that are affected by photoperiod. Decreasing day length stimulates a “dormant” immune system in anticipation of and to counter, the demands and stress of winter, thereby creating a survival advantage.

These changes are thought to be mediated, at least in part, by hormones produced by the pineal and thyroid glands. Also seasonality could be explained by the predominance of warmer, wetter weather providing optimal conditions for *E. coli* O157:H7 growth in the environment or because these times represent periods of increased animal movement between housing and pasture and changes in diet which may lead to stress (Gunn *et al.*, 2007; Vidovic *et al.*, 2007).

To conclude the results found in the present study, give enough indication about epidemiological factors influencing the shedding pattern of *E. coli* O157:H7 in sheep and goats. This could help to develop strategies for pre-harvest preventive and control measures so that the incidence of food borne outbreaks of this food borne pathogen in humans could be reduced to a great extent.

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