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Occurrence of *Escherichia coli* O157:H7 in Faecal Sample of Sheep and Goats in North East Karnataka

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A B S T R A C T

E. coli O157:H7 is an emerging food borne pathogen having zoonotic importance. Though the primary reservoir of the serotype is cattle; sheep and goats are also considered as main reservoir for *E. coli* O157:H7, which act as asymptomatic carriers. Conventional cultural and biochemical methods are time consuming to detect *E. coli* O157:H7 in food borne outbreaks and have less specificity and sensitivity. Latex agglutination test (LAT) tends to be specific, more rapid and reliable. In the present study, faecal samples collected from sheep (n=517) and goats (n=450) from different farms across North Eastern Karnataka during June 2016 to August 2017 were analysed. The samples were processed and analysed for the cultural isolation, biochemical characterisation and latex agglutination test. In this study, LAT was taken as confirmative diagnostic test and the results obtained by cultural isolation and biochemical characterisation were compared with that of LAT to estimate the sensitivity and the specificity. The percent of sheep and goats that showed shedding of *E. coli* O157 in the faeces was 3.67% (19 out of 517) and 2.88% (13 out of 450) respectively. The results obtained show that LAT is a rapid, highly sensitive, species-specific and reliable method for the detection of the pathogenic *E. coli* O157:H7 and could be used for identification and molecular characterisation of *E. coli* O157:H7 in suspected food and water borne outbreaks, disease investigations and routine analysis.

Introduction

The *E. coli* which produce shiga toxins (s*tx)* have been referred as Shiga toxin-producing *E. coli* (STEC). A subgroup of STEC causes watery diarrhoea in humans which can progress to haemorrhagic colitis and potential systemic complications due to the action of s*tx* (Kaper *et al.,* 2004).

These are referred to as EHEC. About 200 EHEC serotypes have been isolated from animal and food sources (Denis *et al.,* 2012). After the first outbreak of bloody diarrhoea due to *E. coli* O157:H7 in Oregon and Michigan, U.S.A. in 1982, and *E. coli* O157:H7 is regarded as the most significant EHEC of humans. From an evolutionary standpoint, pathogenic *E. coli* have diverged from a common ancestor of commensal strains. *E. coli* O157:H7 evolved pathogenic by acquiring virulence factors through plasmids, transposons, bacteriophages and/or pathogenicity islands.

E. coli O157:H7 is a zoonotic bacterium that causes human disease. It is mainly pathogenic to humans (Soderlund *et al.,* 2012) does not cause any clinical disease except diarrhoea (in rare occasions) in cattle and other animals due to the difference in distribution of Gb3 receptors between cattle and humans. The infections by *E. coli* O157:H7 have been reported of increasing frequency from all parts of the world in the form of food poisoning outbreaks (Jo *et al.,* 2004).

E.coli O157:H7 is one of the most important food-borne pathogens, causing diarrhoea, hemorrhagic colitis and haemolytic-uremic syndrome in humans worldwide. Important reservoirs of pathogenic *E. coli* O157:H7 in the environment have been ruminants, particularly cattle, sheep and goats which are asymptomatic carriers. These asymptomatic carriers normally shed the organism in faeces contaminating soil and surface waters. Transmission to people occurs primarily via ingestion of inadequately processed contaminated food or water and less frequently through contact with manure, contaminated soil and water or animals and infected people. Cattle are the major reservoirs of *E. coli* O157:H7 followed by sheep and goats. Because of the severity of illnesses and the apparent low infective dose (Bach *et al.,* 2002), *E. coli* O157:H7 is considered one of the most serious of known food borne pathogens (Blanco *et al.,* 2003). The authority of the Federal Meat Inspection Act, FSIS declared *E. coli* O157:H7 as an adulterant in raw ground beef and enforced "zero tolerance" (USDA-FSIS, 2015).

The pathogen is carried in the intestinal tract and excreted in faeces. The entry of the bacilli into the meat by carcass contamination can be through transfer of pathogen from the intestines during the evisceration procedure, from the hide onto the carcass during flaying, contaminated equipment and tools used during flaying, contaminated operator's hands or contaminated dust particles and water droplets spread by aerosols generated in the production process (Narvaez-Bravo *et al.,* 2013). Consumption of raw or undercooked foods, especially undercooked minced beef and meat has been found to be the most common means of transmission (Chapman *et al.,* 2001).

In India, there is paucity of information on prevalence of *E. coli* O157:H7 in sheep and goats. With this background, the present research work was undertaken to isolate and confirm *E. coli* O157:H7 from faecal samples of sheep and goats by cultural identification, and biochemical characterisation. Further confirmation was done by latex agglutination test.

Materials and Methods

Study area and animals

The study was carried out during from June 2016 to August 2017 and faecal samples were collected from sheep (n=517) and goats (n=450) from different farms across North Eastern Karnataka.

Sample collections

The faecal samples were collected in sterile vials directly from rectum using sterile cotton swab sticks (Hi Media, Mumbai, India). 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 and biochemical characterisation of *E. coli* O157:H7 from faecal samples

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 small, circular colourless colonies (sorbitol negative) with a smoky centre and measuring 1-2 mm in diameter indicated *E. coli* O157:H7 as shown in Fig 1.

The suspected *E. coli* O157:H7 isolates showed Gram negative character indicated by pink coloured coccobacilli (took counter stain safranin) under the 100x oil immersion microscope. When further inoculated on EMB agar differential media, the suspected *E. coli* O157:H7 isolates produced black centered colonies with metallic sheen on EMB agar as shown in Fig 2.

Further, isolated colonies were subjected to various biochemical tests such as IMViC (Indole, Methyl Red, Voges Proskeur and Citrate utilization) tests, Glucuronidase test, Nitrate reduction test, Lysine utilization test, ONPG test and sugar fermentation tests (Lactose, Glucose, Sucrose, and Sorbitol tests) using KB010 *E. coli* identification kit (Hi Media, Mumbai, India). The isolates which showed positive reaction for Indole test, Methyl red test, Glucuronidase test, Nitrate reduction test, Lysine utilization test, ONPG test and sugar fermentation tests (Lactose, Glucose, Sucrose) and negative for Voges-Proskauer test, Citrate utilization test and Sorbitol fermentation tests were presumed as positive for *E. coli* O157:H7 as shown in Figure 3.

For the confirmation of 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 as shown in Fig 4.

Results and Discussion

Domestic and wild animals are source of *E. coli* O157:H7 but the major animal carriers are healthy domesticated ruminants, primarily cattle, sheep and goats which act as asymptomatic carriers (La Ragione *et al.*, 2009). These asymptomatic carriers normally shed the organism in faeces contaminating soil and surface water. Considering the zoonotic importance of *E. coli* O157: H7, rapid method for its detection are important to identify the source of outbreak and to assure public safety.

In India too, there could be food borne outbreaks from this pathogen as people consume meat and meat products. However, systematic diagnosis of food borne outbreaks in India is a rare phenomenon. Few scientists have worked on the incidence of this pathogen in various livestock products (Bindu and Krishnaiah 2010), human patients (Khan *et al.*, 2002a; Khan *et al.*, 2002b) and environmental samples (Hazarika *et al.*, 2007).

In the present study faecal samples were inoculated onto modified Tryptone Soya Broth (mTSB). Tryptone Soya Broth as an enrichment medium specifically for *E. coli* O157:H7 has been recommended by ISO committee. Several researchers from India and other countries have used Tryptone Soya Broth supplemented with novobiocin as enrichment medium for *E. coli* O157:H7 (Bindu and Krishnaiah, 2010; Puttalingamma and Harshvardhan, 2013).

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* (Adamu *et al.,* 2014). Centers for Disease Control and Prevention also recommends Sorbitol-MacConkey 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 often in concentration that inhibits most other *E. coli.*

In the present study, inoculum from the enrichment medium was inoculated onto CT-SMAC which acts as selective and differential medium. Only the typical sorbitol negative colonies were considered as positive for *E. coli* O157:H7. Many other researchers have used CT-SMAC agar as a selective medium for the isolation of *E. coli* O157:H7. Bindu and Krishnaiah (2010) tested 250 samples collected from various sources for the presence of *E. coli* O157:H7 by employing cultural isolation on CT-SMAC agar and reported that only 11 samples were positive by cultural methods. Similarly Aseel *et al.,* (2013) used CT-SMAC agar for isolation of *E. coli* O157:H7 strain from fecal samples of zoo animal with satisfactory results. The isolated sorbitol negative colonies were further subjected for Gram's staining, where in the suspected *E. coli* O157:H7 isolates took counter stain and seen as pink coloured coccobacilli. Further, when inoculated on EMB agar as differential media, the suspected *E. coli* O157:H7 isolates produced black centered colonies with metallic sheen and were presumed to be positive for *E. coli* O157:H7.

Based on the cultural isolation method, per cent animals that showed shedding of *E. coli* O157:H7 was 7.73 in sheep and 6.00 in goats. The results obtained in this study are in correlation with Wani *et al.,* (2003), Oporto *et al.,* (2008), Mersha *et al.,* (2010) and Akanbi *et al.,* (2011).

Conventional culture methods are very useful for the identification of *E. coli* O157:H7. However, these methods are time consuming (Arthur *et al.,* 2005) and there is a possibility to get false positive results (Orth *et al.,* 2009) as supported by the present findings for identification of *E. coli* O157:H7.

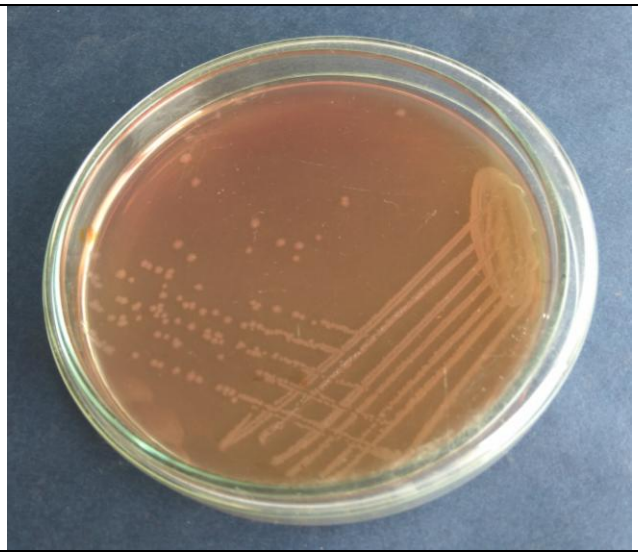

Fig.1 CT-SMAC agar plate showing Sorbitol negative colonies (White coloured) indicating positive for *E. coli* O157:H7

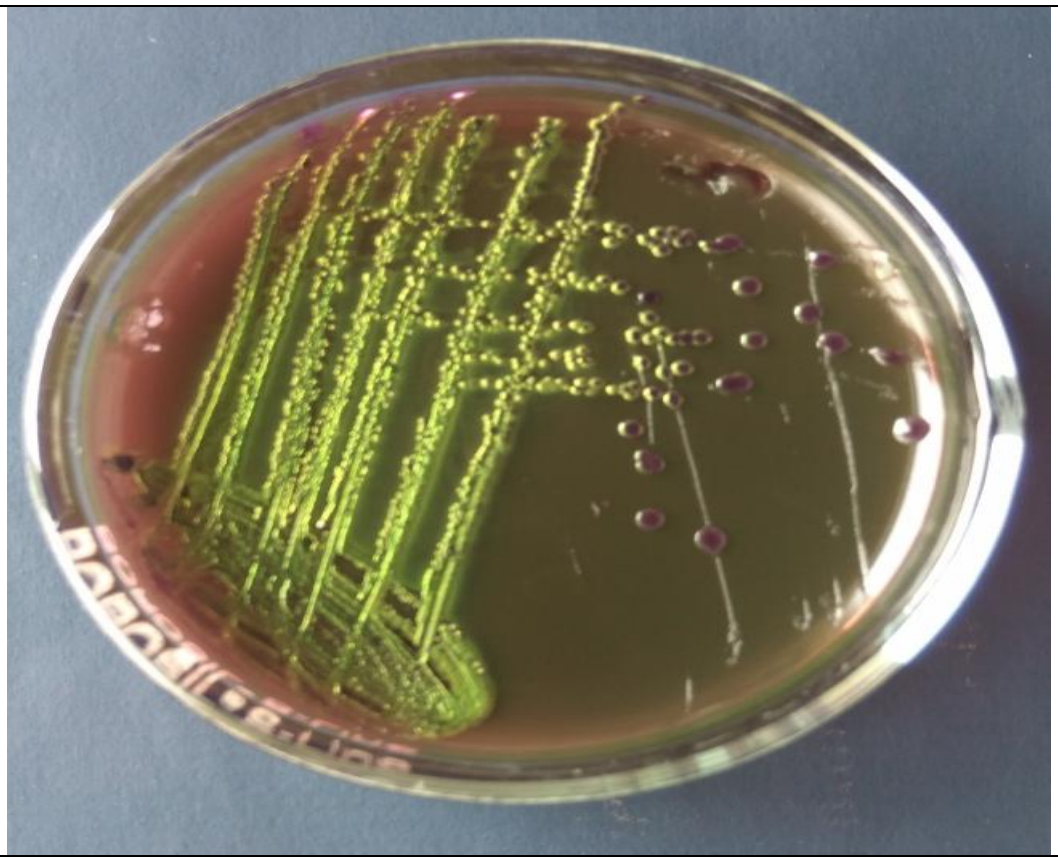

Fig.2 EMB agar showing metallic sheen suspected for *E. coli* O157:H7

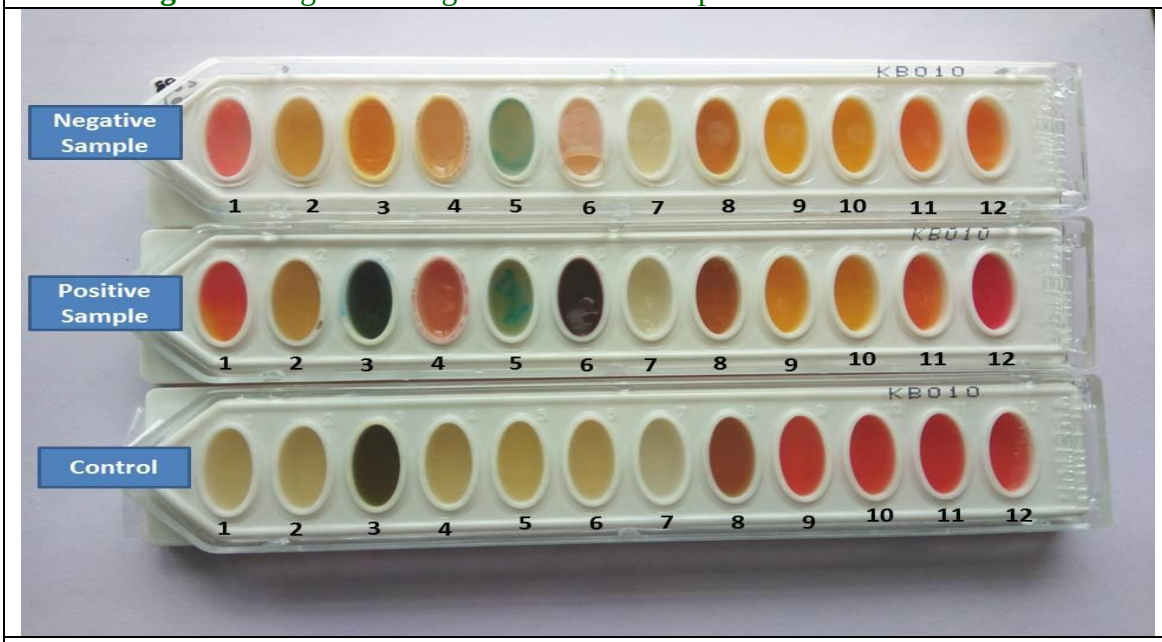

Fig.3 Sample positive for *E. coli* O157:H7 showing biochemical characterization on KB010 *E. coli* identification kit (Hi Media, Mumbai, India)

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

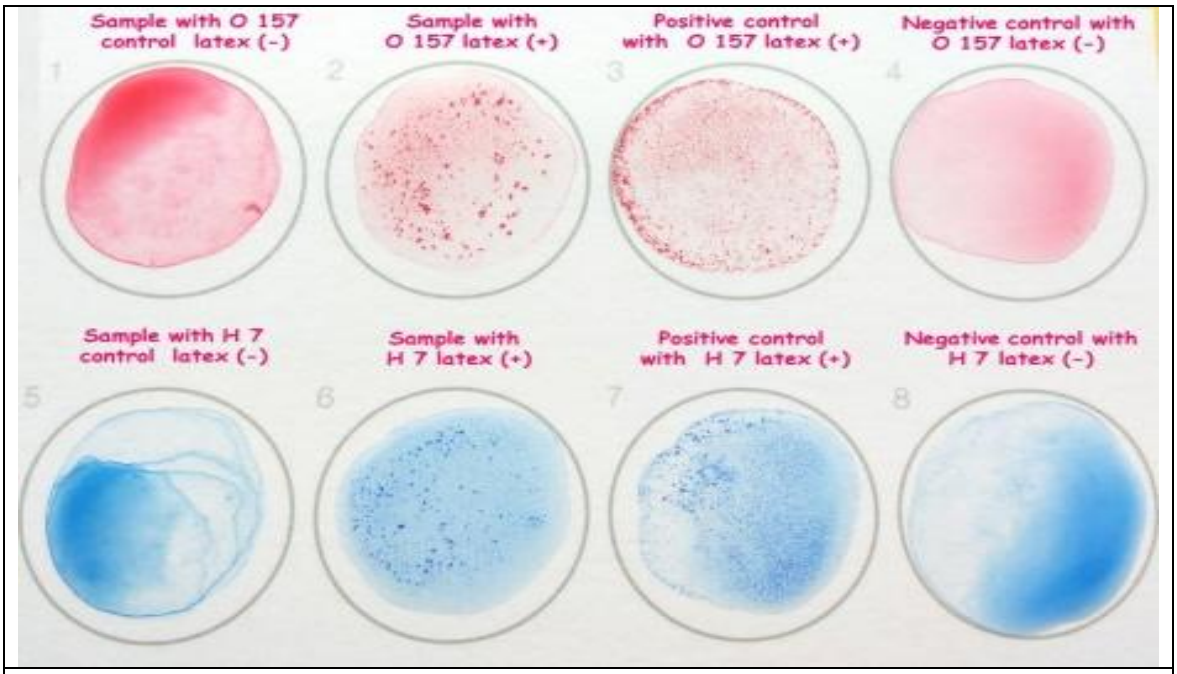

Fig.4 Sample positive for O157 and the H7 antigens showing agglutination reaction in 2nd and 6th circle similar to positive controls, indicating positive for *E. coli* O157:H7.

Table.1 Identification of *E. coli* O157:H7 in the faecal samples of sheep and goats by using different diagnostic methods

Diagnostic test/ Species	Sheep			Goats		
	Positive	**Negative**	**Per cent positive (%)**	**Positive**	**Negative**	**Per cent positive (%)**
Cultural identification	40	477	**7.73**	27	423	**6.00**
Biochemical Characterisation	25	492	**4.83**	16	434	**3.77**
Latex agglutination test	19	498	**3.67**	13	437	**2.88**

As such there are no differences in the biochemical characters between *E. coli* O157:H7 and other serotypes of *E. coli* except for sorbitol fermentation. *E. coli* O157:H7 is sorbitol negative and other serotypes are sorbitol positive (Bettelheim, 2007).

The present study revealed 4.83 % of sheep and 3.77 % of goats as shedders of *E. coli* O157:H7 based on the identification by biochemical characters. Similar results on biochemical tests were obtained by many researchers for detection of *E. coli* O157:H7 (Bindu and Krishnaiah, 2010; Faten and Afaf, 2013; Adamu *et al.*, 2014).

However, biochemical reactions alone cannot differentiate between *E. coli* O157:H7 and other serotypes of *E. coli* (Bettelheim, 2007). Strockbine *et al.*, (1998) mentioned *Escherichia hermanii* is biochemically similar to *E. coli* O157:H7 and can produce sorbitol

negative colonies on CT-SMAC indicating the chances of getting false positive samples.
In this context latex agglutination test (Wellcolex® *E. coli* O157:H7) was used in this study for the confirmative identification of *E. coli* O157:H7 by detecting presence of the O157 and the H7 antigens. The isolates positive for O157 and the H7 antigens showed agglutination reaction with latex coated with respective monoclonal antibodies. 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 (USDA FSIS, 2015).

Based on the results of latex agglutination test 3.67% of the sheep samples and 2.88% of the goats samples showed shedding of *E. coli* O157:H7. Similar type of results was also noticed by many researchers using latex agglutination test for the confirmation of *E. coli* O157:H7 isolates (Mohmmed *et al.,* 2012; Divya *et al.,* 2013 and Faten and Afaf, 2013).

The results of cultural isolation, biochemical Characterisation and Latex agglutination test of *E. coli* O157:H7 from faecal samples of sheep and goats are presented in Table 1.

Out of the total faecal samples tested in the present study, per cent of shedding of *E. coli* O157:H7 in sheep was found to be 3.67% (19 out of 517 samples) and 2.88 % in goats (13 out of 450 samples). So the percentage of sheep showing shedding of *E. coli* O157:H7 in faeces was higher (3.67%) than goats (only 2.88 %). These findings indicated that, though sheep and goats are considered as reservoir for *E. coli* O157:H7 (Ogden *et al.,* 2005; Novotna *et al.,* 2005; Mersha *et al.,* 2010 and Yilmaz, 2014), sheep are more likely to shed this zoonotic pathogen than goats. These results are in accord with the results obtained by Mersha *et al.,* (2010), Akanbi *et al.,* (2011) and Soderlund *et al.,* (2012). But the results are on contrary with Rey *et al.,* 2006. The differences in host response and excretion dynamics could be the reason for such a vast gap in shedding of this pathogen between sheep and goats. There could be differences in the innate immune response among the two species that negates bacterial replication on the epithelium and either reduces attachment to or increases detachment from the epithelium of the terminal rectum (Mersha *et al.,* 2010).

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