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Prevalence and virulence gene profiles of Shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* from diarrhoeic and healthy lambs in India

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Abstract

This communication describes the prevalence and virulence attributes of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolates in lambs with and without diarrhoea in Kashmir, India. One hundred twenty and 164 *E. coli* isolates belonging to 56 different 'O' serogroups were isolated from 101 diarrhoeic and 135 healthy lambs, respectively. All the 284 isolates were screened for presence of stx_1, stx_2, *eae* and *ehxA* genes using multiplex polymerase chain reaction (m-PCR). Forty four (36.67%) isolates from lambs with diarrhoea carried at least one virulence gene studied. Twenty one (17.5%) and 15 (12.5%) isolates from diarrhoeic lambs were STEC and EPEC, respectively. Thirty (18.3%) isolates from healthy lambs possessed at least one virulence gene studied. Fourteen (8.53%) and 16 (9.75%) isolates from healthy lambs were detected as STEC and EPEC, respectively. Sixteen (45.71%) of 35 STEC isolates from both diarrhoeic and healthy lambs, carried both stx_1 and stx_2 genes, 15 (42.85%) had stx_1 alone and four (11.42%) had stx_2 gene alone. Statistically the difference between prevalence of STEC in diarrhoeic and healthy lambs was significant but that of EPEC was insignificant. One (2.85%) and 28 (80%) of STEC isolates possessed *eae* and *ehxA* genes, respectively. The high percentage of STEC and EPEC prevalent in diarrhoeic and healthy lambs may serve as source of infection to humans. STEC seem to be associated with diarrhoea in lambs.
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Keywords: Diarrhoea; EPEC; Healthy; Lambs; Multiplex-PCR; STEC

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) are commonly recovered from the faeces of food-producing animals and pose threats to health of humans and livestock (Nataro and Kaper, 1998). STEC cause serious gastrointestinal and systemic diseases such as haemorrhagic colitis (HC) and haemolytic-uraemic syndrome (HUS), while EPEC isolates are leading cause of diarrhoea, especially among infants in the developing world (Paton and Paton, 1998a). EPEC isolates are defined as intimin-containing diarrhoeagenic *E. coli* isolates that possess the ability to form attaching and effacing (AE) lesions on intestinal cells and that do not possess genes coding for Shiga tox-

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ins (Kaper, 1996). The pathogenicity of STEC and EPEC is mainly mediated by Shiga toxins (Stx1, Stx2 and their variants) encoded by stx_1 and stx_2 genes and the products of the locus of enterocyte effacement (LEE) pathogenicity island, with the *eae* gene that encodes for the intimin protein involved in the intimate adhesion of bacteria to enterocytes and production of AE lesion on the intestinal mucosa (Paton and Paton, 1998b). Besides, a specific plasmid encoded haemolysin called EHEC haemolysin, which is encoded by *ehxA* gene, might contribute to the virulence of STEC for humans (Beutin et al., 1995).

Little is known about the role of STEC in the diarrhoea of lambs due to, among several factors, the limited number of epidemiological studies carried out on STEC isolates from diarrhoeic lambs (Blanco et al., 1996; Munoz et al., 1996). Previously, we reported isolation and characterization of a limited number of STEC and EPEC isolates from lambs with diarrhoea in India (Wani et al., 2003). The present study is a comprehensive attempt to investigate the *E. coli* isolates from lambs with diarrhoea vis-a-vis healthy lambs for the presence of stx_1, stx_2, *eae* and *ehxA* genes as a possible cause of the disease in them as well as a possible source of infection to humans. The present study is also a first report of isolation and characterization of STEC and EPEC from healthy lambs in India.

2. Materials and methods

2.1. Sampling and isolation of E. coli isolates

During November 2002 to January 2004, 236 faecal samples from 0 to 3-month-old crossbred lambs were collected from different organized farms and veterinary clinical premises. Out of these 101 samples were from lambs with diarrhoea, while 135 were from healthy lambs. The samples were collected by rectal swabbing and transferred to laboratory on ice.

Faecal samples were immediately inoculated on MacConkey's agar (HiMedia, Mumbai, India) plates. After overnight incubation at 37 °C, two to three rose pink colonies per plate were randomly picked up and subcultured onto eosin methylene blue (EMB) agar plates to observe the characteristic metallic sheen of *E. coli*. The well-separated colonies were picked up on nutrient agar slants as pure culture and subjected to standard morphological, biochemical tests as described by Collee et al. (1996). Altogether, 284 (120 from diarrhoeic and 164 from healthy lambs) isolates were identified as *E. coli*.

2.2. Serogrouping

The *E. coli* isolates were serogrouped on the basis of their O antigen by National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, H.P.173204 (India).

2.3. Extraction of bacterial DNA

Bacterial isolates were grown in nutrient broth (HiMedia) at 37 °C overnight. Organisms from 1.5 mL growth were pelleted by centrifugation at 1200 × *g* for 10 min. The bacterial pellet was resuspended in 150 μL of sterile distilled water. The bacteria were lysed by boiling for 10 min in a water bath. The lysate was centrifuged again as before and the supernatant was used directly as template for PCR.

2.4. Detection of virulence genes by multiplex PCR

E. coli isolates were subjected to multiplex polymerase chain reaction (m-PCR) for detection of stx_1, stx_2, *eae* and *ehxA* genes. Multiplex PCR was performed with Gene Amp PCR System 2400 thermal cycler (Applied Biosystems, USA). Oligonucleotide primers were procured from M/S Sigma GENOSYS. The primers and the predicted lengths of PCR amplification products are listed in Table 1.

PCR assays were carried out in a 25 μL volume as per the method of Paton and Paton (1998b) with slight modifications. Samples were subjected to two regimes of amplification. First regime consisted of 15 cycles. Each cycle consisted of 1 min of denaturation at 95 °C, 2 min of annealing at 65 °C and 1.5 min of extension at 72 °C. The second regime consisted of 20 cycles of 1 min of denaturation at 95 °C, 2 min of annealing at 60 °C and 2 min of extension at 72 °C. This was followed by final extension of 5 min at 72 °C.

Amplified PCR products were analysed by gel electrophoresis in 2% agarose containing ethidium bromide (0.5 μg/mL) (Sambrook and Russel, 2001). The products were visualized with UV illumination and imaged with gel documentation system (GDS 8000 system, UVP, UK).

2.5. Statistical analysis

The data on prevalence of STEC and EPEC strains in diarrhoeic and healthy lambs was analysed by χ^2 test.

3. Results

A total of 284 *E. coli* isolates were isolated from 236 feacal samples. Out of 120 *E. coli* isolates from lambs with diarrhoea, 94 belonged to 36 serogroups, 18 isolates were untypeable and remaining eight were rough, as shown in Table 2. Similarly out of 164 *E. coli* isolates from healthy lambs 135 belonged to 33 serogroups, 21 were untypeable and eight were rough, as shown in Table 2.

Out of 120 *E. coli* isolates from lambs with diarrhoea, 44 (36.67%) isolates carried at least one virulence gene studied. Twenty one (17.5%) and 15 (12.5%) isolates were detected as STEC and EPEC, respectively. STEC isolates belonged to 12 different serogroups, while as ten EPEC isolates belonged to five different serogroups and

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