PREVALENCE, SEROTYPES AND PATHOGENICITY OF *ESCHERICHIA COLI* ASSOCIATED WITH DIARRHOEA IN DAIRY CALVES

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ABSTRACT

Sixty two (53.44%) strains of *E. coli* were isolated from 116 fecal samples collected from cow and buffalo calves suffering with diarrhoea. Biochemical characterization revealed 9.6% atypical strains, which were weakly urease positive. The isolates of *E. coli* were typed into 27 different 'O' serotypes. 4 strains were untypable, while 2 were rough. Whole bacterial cultures as well as cell free filtrate of randomly selected *E. coli* strains of O2, O14, O15, O28, O60, O69, O138, and 0171 serotypes and one untypable strain caused mortality in mice on intraperitoneal inoculation.

KEY WORDS: Calves, Diarrhoea, *Escherichia coli*, Serotypes

INTRODUCTION

Neonatal diarrhoea is one of the main causes of calf morbidity and mortality resulting in major economic losses to the dairy and beef herds. Mortality in neonatal calves has mostly been attributed to infectious agents. *Escherichia coli,* an important pathogen of bovine neonates, is established in intestines shortly after the birth and remains throughout life. Most of the strains of *E. coli* are harmless saprophytes, but a few virulent strains cause a variety of intestinal and extraintestinal infections (Radostits *et al.*, 2000). Several *E. coli* serotypes, causing morbidity and mortality, have been isolated from calves suffering with diarrhoea (Wani *et al*., 2003; Mahajan *et al*., 2013 and Udayankar and Sharda, 2009). The infected animal might also act as reservoirs of certain *E. coli* strains pathogenic for man (Fairbrother and Nadeau, 2006). Keeping in view the economic and public health importance of enteric colibacillosis, the present study was undertaken to study the prevalence of *E.coli* in dirrhoeic calves. Biotyping, serotyping and pathogenecity of the isolated strains of *E.coli* was also carried out.

MATERIALS AND METHODS

One hundred sixteen faecal samples were aseptically collected in sterile sample bags (Hi Media) from 63 buffalo and 53 cow calves suffering with diarrhoea. These animals were reared both in the organized and unorganized farms.

Isolation, Biotyping and Serotyping

The faecal samples were processed for isolation of *E. coli*, following the method of Edwards and Ewing (1972). For isolation, MacConkey broth (single strength), MacConkey agar (MCA) and Eosin-methylene blue (EMB) agar were used as an enrichment, differential and selective medium, respectively. The enrichment was done at 37°C for 12-18 h, while the MCA and EMB agar were incubated for 24 h. The smooth and moist colonies with metallic sheen on EMB agar were randomly subcultured and purified by limiting dilution. The isolates were identified as *E. coli* on the basis of their cultural, morphological and biochemical characteristics (Barrow and Feltham, 1993). They were serotyped at the National Salmonella and Escherichia Typing Centre, Central Research Institute, Kasauli (Himachal Pradesh), India.

Mouse lethality test

Pathogenecity of randomly selected 27 strains each representing one of the serotype, and the 2 rough and 4 untypable strains, were tested in mice as per the procedure of Boro *et al.* (1983). For each strain, a group of 5 Swiss albino mice of 6 week of age were used. Brain heart infusion (BHI) broth grown culture (0.2ml) of each strain containing approximately 2×10^9 organisms/ml was injected intraperitoneally (i.p.) into each mouse and observed up to 96 hours post inoculation. Mice died during the period of observation were necropsied and reisolation of the organisms was attempted from internal organs and heart blood.

Toxicity assay

The cell free filtrate (CFF) of representative strains was prepared as per the procedure of Gianella (1976) and 0.1 ml of each of these was inoculated in adult Swiss albino mice by intra-venous route. The mice were observed up to 72 hours post inoculation.

RESULTS AND DISCUSSION

E.coli was recovered from 62 out of 116 faecal samples collected from cow and buffalo calves suffering with diarrhea. Thus the overall prevalence rate of enteric colibacillosis in the present study was 53.44%, which is in corroboration of 59.2% reported by Dadawala *et al*. (2012). On the contrary, Wani *et al.* (2003), Himanshu and Pal (2012) and Mahajan *et al*. (2013) reported 45.38, 79.17 and 90.0 per cent prevalence rate of *E.coli* in diarrhoeic calves respectively from different regions of India. A higher prevalence rate was recorded in cow calves (66.03%) than in the buffalo calves (42.85%), which is contradictory to the findings of Dadawala *et al*. (2012). The prevalence of enteric colibacillosis in calves is affected by several factors.

In the present study a higher incidence was recorded in male calves (58.33%) than female calves (51.25%). Susceptibility was also evaluated for the incidence in calves of different age groups. The results showed that animals were more susceptible during first two months of age (65.57%) as compared to older calves (40.0%).

Biochemical characterization revealed that all isolates were positive for catalase, methyl red, indole production, nitrate reduction, β-galactosidase activity and decarboxylation of lysine and ornithine, but negative for oxidase, Voges-proskauer, H_2S production and gelatin hydrolysis thereby confirming the typical *E.coli*. However, 6 (9.6%) isolates were weakly positive for urease. Mercado *et al.* (2004) and Arya *et al.* (2008) also isolated urease positive atypical strains of *E. coli* from diarrhoeic calves. Such atypical biochemical behaviour may serve as an important marker or diagnostic tool for epidemiological surveys to trace the source of infection in disease outbreaks.

Sugar fermentation reactions showed that none of the isolate could utilize inositol, whereas all of them fermented arabinose, dulcitol, glycerol, glucose, lactose, mannitol, rhamnose, sorbitol, sucrose and xylose. Sugar fermentation reactions biotyped *E.coli* isolates from diarrheic cow and buffalo calves into 9 and 13 types, respectively (Dadawala *et al*., 2012). Mahajan *et al*. (2013) differentiated 81 isolates of *E.coli* into 52 biotypes on the basis of sugar fermentation. Lactose, xylose and dextrose were fermented by all the isolates, whereas variable results were obtained for sorbitol (92.59%), maltose (90.12%), mannose (90.12%), sucrose (81.48%), mannitol (80.24%) and inositol (13.58%).

Fifty six (90.32%) isolates were typed into 27 different 'O' serogroups; 4 (6.45%) strains could not be typed and 2 (3.22%) were rough strains. Amongst the typable isolates, maximum belonged to group O14, O22 and O138; followed by O88, O20, O60 O116, O3, O13, O28, O69, O106, O110, O127 O159, O2, O12, O15, O97, O98, O105, O108, O132, O149, O166, O171, and O172. Serogroup O22 was also predominantly isolated from diarrheic cow calves by Dadawala *et al*. (2012). Serogroups of *E.coli* detected from diarrheic cow and buffalo calves were also reported by various workers from different parts of India and abroad (Wani *et al.,* 2003; Mercado *et al.,* 2004;

Cookson *et al.,* 2006; Dadawala *et al*., 2012; Himanshu and Pal, 2012 and Mahajan *et al*., 2013). The distribution of different serotypes of *E.coli* varies with geographical regions and their prevalence in man and animals in a particular area.

Whole bacterial culture of *E. coli* strains belonging to the serogroup O2, O14, O15, O28, O60, O69, O138, and 0171 and one untypable strain caused mortality in mice on intraperitoneal inoculation. Congestion of internal organs and white necrotic foci on liver were observed on necropsy. *E. coli* was isolated in pure culture from heart blood and visceral organs of dead mice. Intravenous admininstartion of CFF of isolates also resulted in death of mice with accumulation of excessive fluid in the intestines. Dubey and Sharda (2001) and Yadav *et al.* (2006) also reported identical pathology of certain strains of *E. coli*.

The strains of *E. coli* isolated in this study belonging to serogroups O108, O138 and O149 have been associated with severe disease in humans (Kapoor *et al.,* 1995; Fairbrother and Nadeau (2006) in other parts of globe. There is every possibility of transmission of *E.coli* serotypes from animals to man. Thus the present study suggests to carry out of such investigations in humans with diarrhoeal disease to determine the zoonotic significance of theses strains.

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