DETECTION OF VEROTOXIN PRODUCING STRAIN OF E. COLI IN BUFFALO CALVES

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ABSTRACT

Production of verotoxin (Shigatoxin) is the most important criteria for the detection of enterohaemorrhagic E. coli. There is a paucity of data about the prevalence, burden of disease and complications associated with verocytotoxigenic Escherichia coli (VTEC) in animals in India. The main reservoir for EHEC is the feces of cattle, sheep and goats. In the present studies the verotoxin rapid test for the detection of verotoxin was performed for all the bacterial isolates obtained from faeces of buffalo calves. It is an immunochromatographic rapid test utilizing monoclonal antibodies which are labeled by red colored gold particles. The faecal sample was collected directly from the rectum of each calf by using sterile swabs. The microorganism was cultured on MacConkey agar and eosin methylene blue agar medium for preliminary characterization. On MacConkey agar lactose fermenting smooth colonies and on EMB agar dark centered blue black colonies with metallic shine were identified as E. coli. Dacron swabs were taken from the 32 isolates of E. coli on MacConkey agar plates obtained from diarrhoeic buffalo calves. The swab was suspended in 0.5 ml distilled water containing 50 μg/ml polymyxin B to enhance the release of toxin and the mixture was incubated for 30 minutes at 35°C. Using a micropipette 200 μl of the sample was dispensed into the circular sample port of the test device. The reading was taken after 10 minutes incubation at room temperature. In a positive reaction a distinct red line appears on test zone. Out of 32 isolates of E. coli, only 19 (59.37%) showed a positive reaction for presence of verotoxin.

Keywords: calf, diarrhoea, E. coli, verotoxin

INTRODUCTION

Enterotoxigenic Escherichia coli are probably the most common single cause of undifferentiated neonatal diarrhea in calves. Escherichia coli have several virulence attributes that result in disease in animals. Principally, these promote colonization or adhesion to the mucosa, cause metabolic dysfunction or death of enterocytes, affect the local or systemic vasculature or promote invasion and septicemia. The enteropathogenic strains of E. coli which colonize the mucosa of the small intestine may also secrete cytotoxins (Shigatoxins=verotoxin) that have an effect locally or systemically and are categorized as verotoxin producing (VTEC) or enterohaemorrhagic (EHEC). These are a serious cause of food-borne illness in humans and haemorrhagic enterolitis in calves (Gyles, 2007). Production of verotoxin is the most important criteria for the detection of this group
of bacteria. There is a paucity of data about the prevalence, burden of disease and complications associated with verocytotoxigenic Escherichia coli (VTEC) in animals in India (Gopinathan et al., 2005). This study was undertaken to detect the presence of verotoxin producing Escherichia coli in feces of buffalo calves.

**MATERIALS AND METHODS**

The faecal samples were collected directly from the rectum of each buffalo calf by using sterile swabs. Direct fecal smears were observed and samples were further processed for bacterial culture. The micro-organism was cultured on MacConkey agar and Eosin methylene blue agar medium for preliminary characterization. A total of 32 isolates of E. coli were obtained. The Duopath Verotoxin rapid test was used for identification of enterohaemorrhagic E. coli infections. The Duopath Verotoxin test is an immunochromatographic test utilizing monoclonal antibodies which are labeled by red-colored gold particles. The test device has a circular sample port and an oval-shaped test (VT1, VT2) and control (C) window.

The confluent growth area of the culture from MacConkey agar plates obtained from 32 diarrhoeic buffalo calves. Out of these only 19 (59.37%) showed a positive reaction for presence of verotoxin. A number of studies have been conducted to determine the incidence of verotoxic E. coli in cattle. Khurana and Kumar (2005) conducted studies to determine the status of dairy cattle from Hisar and surrounding areas as the principal reservoir of verotoxic E. coli by faecal examination of healthy and diarrhoeic cow and calves, They observed a significant prevalence of VTEC in faecal samples of both diarrheic as well as healthy cattle. The workers expressed concern that healthy animals in close proximity to humans, manual handling of faeces and poor hygiene at the time of milk production and collection may pose a threat to human health. Rugbjerg et al., (2003) stated

**RESULT AND DISCUSSIONS**

In direct smear examination of rectal swab after staining Gram positive bacilli were seen in few cases of diarrhoeic buffalo calves. On MacConkey agar lactose fermenting smooth colonies and on EMB agar dark centered blue black colonies with metallic shine were identified as E. coli. For epidemiological or clinical purposes E. coli strains are often selected from MacConkey agar plates after presumptive visual identification of lactose fermenting pink colonies. Earlier workers (Duffy, 2003; Paul et al., 2010) have also used similar methods for isolation of E. coli from faeces of animals. There are no specific media recommended for isolation of enterotoxigenic E. coli but like other E. coli strains they can grow on nutrient agar, MacConkey lactose agar, and eosin methylene blue (EMB) agar. Blood agar plates can also be used for isolation of diarrhoeagenic E. coli (Edwards and Ewing, 1972).

Swabs were taken from the E. coli culture of MacConkey agar plates obtained from 32 diarrhoeic buffalo calves. Out of these only 19 (59.37%) showed a positive reaction for presence of verotoxin. A number of studies have been conducted to determine the incidence of verotoxic E. coli in cattle. Khurana and Kumar (2005) conducted studies to determine the status of dairy cattle from Hisar and surrounding areas as the principal reservoir of verotoxic E. coli by faecal examination of healthy and diarrhoeic cow and calves, They observed a significant prevalence of VTEC in faecal samples of both diarrheic as well as healthy cattle. The workers expressed concern that healthy animals in close proximity to humans, manual handling of faeces and poor hygiene at the time of milk production and collection may pose a threat to human health. Rugbjerg et al., (2003) stated
that among calves aged 1-4 months old, carriage of VTEC E. coli O157 was reduced if the calf had suckled colostrums from the mother or if the calf had stayed more than 2 days with the mother after calving. Although bovine VTEC belong to a large number of O serogroups it is not known whether all variants of VTEC are equally pathogenic as verotoxin production alone may not be sufficient for VTEC to cause disease. A factor that may affect virulence of VTEC is the ability to cause attaching and effacing (AE) lesions in the intestinal mucosa. Little is known about the putative pathogenic role of VTEC in cattle. Looking into the present finding of a high incidence of VTEC in diarrhoeic calves but negligible incidence of VTEC non-diarrhoeic animals it can be suggested that contrary to the earlier variable reports VTEC may be pathogenic to calves causing diarrhea.

REFERENCES


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