Investigation of some virulence factors associated with E. coli isolated from diarrheic buffalo calves

F. M. Ghanem[1], M. N. El-Sheery[1], K. M. Ibrahim[1], A. M. El-Sherif[2]

[1]*Department of Veterinary Medicine, Faculty of Veterinary Medicine Suez Canal University*
[2]*Deptment of Veterinary Medicine, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef , Egypt*

In this study a total of 120 diarrheic buffalo calves were examined clinically and bacteriologically was investigated. The role of *E. coli* in diarrheic buffalo calves. *E. coli*, could be isolated from 31 (25.80%) calves. K_{99} antigen could be detected in (12.90%) isolates. Studying some virulence factors of *E. coli* isolates revealed that 28 (90.30) isolates showed congored binding, 29 (93.50%) isolates were able to survive in serum and 23 (74.19%) were able to grow in calf serum, 25 (80.64 %) isolates could be proved as enterotoxin producers and caused accumulation of fluids in the intestinal tract of the inoculated mice. In addition, 28 (90.30 %) were able to produce verotoxins. The present study demonstrated the correlation between the presence of different virulence factors in *E. coli* isolates and its pathogenicity to newborn calves and its role in diarrheic calves.

Samples. Fecal swabs were collected from the rectum of 120 diarrheic buffalo calves.

Media. Different media were used for isolation and identification of *E. coli*. These include neutrient broth (Oxoid), nutrient agar (Oxoid), blood agar (Difco), MacConkey's agar (Oxoid) and eosin methylene blue (Oxoid). Media for congo red binding activity, serum resistance and verocytotoxicity test were obtained from (Oxoid). Medium for enterotoxin assay was obtained from (Difco).

Antisera. K_{99} antisera were prepared in rabbits using standard K_{99} antigen. Diagnostic *E. coli* antisera (O) and (K) were obtained from (Welcom), Calves serum was used for serum resistance test.

Vero cells. (green monkey kidney cells) were used in verocytotoxicity test.

Identification of *E. coli* isolates. It was carried out according to (Cruickshank *et al*. 1975 and Konemann *et al.,* 1993).

Serological typing of *E. coli* agglutination test. It was carried out according to (Edwards and Ewing 1972).

Congo red binding test. It was carried out according to (Berkhoff and Vinal 1986).

Enterotoxin assay using infant mouse test. It was carried out according to (Robins-Brown *et al.,* 1993).

Serum resistance test. It was carried out according to (Barrow and Hill 1989).

Cytotoxicity assay. It was carried out according to (Emery, *et al*., 1992).

Enteritis in newborn calves still represents a serious problem for the livestock production resulting in severe economic losses due to high morbidity and mortality, (Adesiyun *et al.*, 2001). Control of calf diarrhea is usually a difficult task due to the multifactorial nature of the problem including the immunological and nutritional status of newborn calves, environmental conditions and involvement of different types of etiological agents (Aly and Sohair 1999).

Pathogenic strains of *E. coli* are considered the major cause of neonatal diarrhea (Khan and Khan 1997), due to the combination of different virulence factors including the ability to produce enterotoxins, vesotoxins and the expression of specific adhesions that enable the organism to colonize the intestinal epithelium.

The present study was carried out to investigate the role of pathogenic *E. coli* in diarrheic buffalo calves as well as the virulence factors associated with *E. coli* isolated from clinical cases including Congo red binding activity, serum resistance, enterotoxigenicity using infant mouse assay and cytotoxicity using vero cells.

Material and Methods

Animals.

Calves. A total of 120 diarrheic buffalo calves were used in this study.

Mice. 1-4 days old Swiss albino white suckling mice of were used for detection of entero-toxigenic activity of *E. coli* isolates.

Results and Discussion

Table (1): Results of *E. coli* isolation from diarrheic calves.

No. of calves	No. of isolates		K^{99}+
	1-14 days	15-30 days	
120	24 (20%)	7 (5.84%)	4 (12.90%)
Total	31(25.80%)		

Table (2): Serotyping of *E. coli* isolates.

Serotypes	Number
055 K_{59}	16
026 K_{60}	7
0125 K_{70}	4
0114 K_{90}	2
0128 K_{67}	1
0111 K_{58}	1
Total	31

Table (3): Detection of some virulence factors of *E. coli* isolates.

No. of isolates	Virulence factors	Positive strains
31	Congo red binding	28 (90.30%)
	Survival in calf serum	29 (93.50%)
	Growth in calf serum	23 (74.19 %)
	Heat stable toxins	25 (80.60 %)
	Vero cells cytotoxicity	28 (90.30%)

E.coli isolates revealed the presence of 6 serotypes, 055 K_{59} (16 isolates), 026 K_{60} (7 isolates), 0125 K_{70} (4 isolates), 0114 K_{90} (2 isolates), 0128 K_{67} (one isolate) and 0111 K_{58} (one isolate), Table (2). Similar serotypes were reported by Adel *et al.* (1987) from buffalo calves that indicate the high prevalence of such serotypes and magnify their role in development of enteritis among buffalo calves. The results of Congo red binding of *E. coli* isolates revealed that 28 (90.30%) were positive (Table 3). Such finding indicated a positive correlation between the isolation of congo red positive *E. coli* and the development of enteritis as reported by (Styles and Flamer 1991).

Results of serum resistance of *E. coli* isolates revealed that 29 (93.50%) survived in calf serum and 23 (74.19%) were able to grow in calf serum. The ability of *E. coli* organism to survive and grow in serum in spite of the bactericidal activity of complement has been

Discussion

The present study was carried out on 120 buffalo calves that showed variable degrees of diarrhea, depression and dehydration.

Bacteriological examination of fecal swabs of buffalo calves, revealed isolation of *E. coli* from 31 (25.80 %) calves including 24 of 1-14 days old and 7 of 15-30 days old (Table 1). Such findings agreed with that reported by (Adesyuen *et al*. 2001) who concluded that the prevalence of infection is higher in younger than older calves. They added that diarrhea resulted from *E. coli* infection is the outcome of interplay between the pathogen and the status of immunity at this age. In order to establish a good status of immunity, Larsson, (1995) recommended that Calf should take 2 kg colostrum from the first milking within five hours after birth. K_{99} antigen is responsible for adhesion of the organism to the intestinal mucosa of diarrheic calves, (Yadav and Bhatia 2002). Serological typing of the 31

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considered as an important attribution to its pathogenicity. It is also suggests the possibility of its multiplication in blood, (John, *et al*., 1989).

In this study 25 isolates (80.60%) were proved to be enterotoxin producers and caused accumulation of fluids in the intestinal tract of inoculated mice. This proved high correlation between isolation of enterotoxigenic *E. coli* and induction of diarrhea. Such finding is supported by (Al Majali *et al.*, 2000 and Tanois *et al*., 2000) who concluded that pathogenic *E. coli* usually causes diarrhea either by elaboration of enterotoxins or invasion of the intestinal mucosa.

Among the 31 isolated *E. coli* strains, 28 (90.30%) were able to produce verotoxins and were pathogenic to vero cells, (Table 3). This was in agreement with that reported by (Ball et al., 1994).

In conclusion the present study demonstrated the correlation between the presence of different virulence factors in *E. coli* isolates including congored activity, serum resistance, enterotoxin production and verotoxin activity and its pathogenicity in newborn calves. The results also indicated that *E. coli* continous to be a major problem causing a high morbidity and mortality leading to severe economic losses among calves. Due to the increased prevalence of *E. coli* infection among calves, it is recommended to establish a good passive protection in enzootic areas, which can be achieved by feeding calves on milk from immunized dams.

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