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Isolation and pathogenic attributes of *Escherichia coli* isolates from diarrhoeic foals

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Foal mortality in equine farms may be up to 20% and diarrhoeal diseases continue to be the leading cause of morbidity and mortality in foals below 6 months of age. *Clostridium*, *Salmonella*, *Escherichia coli,* rotavirus and *Cryptosporidium* are the major aetiological agents associated with foal diarrhoea (Conner and Darlington 1980, Netherwood *et al*. 1996, Holland *et al*. 1996, Weese *et al*. 2001). *E. coli* is often found associated with several human and animal ailments (Senior *et al.* 1992, Kapur *et al.* 1992). This is particularly true in compromised animals under the stressful conditions. Although *E. coli* strains belonging to serogroups O: 157, O: 26 and O: 128 are considered pathogenic (O'Brian *et al.* 1982), their pathogenicity depends largely on their pathogenic attributes. Their association with diarrhoea in domestic animals is established (Sharma *et al*. 1992). However, information on pathogenic attributes of the isolates of *E. coli* from equines is lacking. The present communication reports the isolation of *E.coli* strains from apparently healthy and diarrhoeic foals with their antibiogram and some pathogenic attributes.

Faecal samples or rectal swabs of diarrhoeic foals were collected from foals below 2 months of age from at organized stud farms in Uttar Pradesh and Haryana. Consistency and colour of stool samples were recorded. The samples were transported on ice and the swabs were brought to the laboratory in modified amies transport medium with charcoal and kept at room temperature till processing for isolation was done. For isolation, buffered peptone water, modified McConkey lactose agar (MLA) and Eosin-methylene blue (EMB) agar were used as enrichment, differentiation and selective media, respectively. The smooth, moist colonies having metallic sheen on EMB agar were randomly picked up and processed for isolation and identification. The isolates were identified on the basis of their cultural, morphological and biochemical characteristics (Cowan and Steel 1975). These were serotyped at the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli (Himachal Pradesh), India. Antibiogram of all isolates were determined for 23 antimicrobial agents, namely ampicillin (A), amikacin (Ak), amoxycillin (Am), azithrocin (At), chloramphenicol (C), clindamycin (Cd), ciprofloxacin (Cf), erythromycin (E), enrofloxacin (Ex), gentamicin (G), kanamycin (K), lincomycin (L), neomycin (N), nalidixic acid (Na), norfloxacin (Nx), oxytetracyline (O), penicillin (P), polymixin B (Pb), tobramycin (Tb), tylosin (Tl), trimethoprim (Tr), sulphadiazine (Sz), using disc diffusion method (WHO 1961).

To examine the cytotoxigenicity of isolates, the cell free culture filtrates were prepared (Evans *et al.* 1973). These were tested on monolayer of Vero cell line at 37°C and observations were recorded after 18 h, 24 h, 42 h, 48 h and 66 h of incubation as under:

- `-` : No change Negative
- `+` : Slight rounding (conglomeration) Doubtful
- `++` : Rounding and detachment started (25%) Slightly cytotoxigenic (verotoxigenic)
- `+++` : Detachment to 50–75% Highly cytotoxigenic (verotoxigenic)
- `++++` : Maximum detachment Extensively cytotoxigenic (verotoxigenic)

The heat stable *E. coli* enterotoxin (ST) in culture supernatants was detected by competitive enzyme immunoassay employing the *E. coli* ST EIA kit as per manufacturer's directions, to examine the enterotoxigenicity of isolates.

Pathogenicity of the isolates was examined by mice-lethality test. The isolates were propagated in BHI broth and 0.1 ml of each of 6 h-old-culture was administered intra-peritoneally in adult mice (1½ –10 month-old). The animals were observed regularly for 72 h for any change. Mice found dead during this period were opened and lesions were observed. The mice which were depressed and having diarrhoea were considered morbid.

Isolates (73) of *E. coli* were isolated from 107 faecal samples or swabs or rectal swabs obtained from diarrhoeic foals. No *Salmonella* or clostridia could be isolated from these samples. These *E. coli* isolates belonged to 36 different

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Table 1. Sample collection and isolates obtained from them

Sample consistency	Farm in Haryana		Farm in UP		Total	
	No. of samples (isolates)	%	No. of samples (isolates)	%	No. of samples (isolates)	%
Normal	5 (3)	60.00	9 (3)	33.33	14 (6)	42.86
Loose	24 (15)	62.50	32 (24)	75.00	56 (39)	69.64
Watery	11 (5)	45.45	26 (23)	88.46	37 (28)	75.68
Total	40 (23)	57.50	67 (50)	74.63	107 (73)	68.22

Table 2. Cytotoxigenicity of *E. coli* isolates obtained from diarrhoeic foals

Cytotoxigenicity on vero cells	Isolates			
	normal faeces	loose faeces	watery faeces	Total
Negative	2	27	18	47
Doubtful	1	3	4	8
Slightly cytotoxigenic	3	6	6	15
Highly cytotoxigenic	0	3	0	3
Total	6	39	28	73

serogroups, in addition to 6 untypable and 5 rough strains (Table 1). The most common serogroup was O: 101 (10) followed by O: 9 (5), O: 91 and O: 8 (4 each) while 3 each belonged to serogroups O: 6 and O: 156, two each to O: 60, O: 117 and O: 141. Rest 27 isolates belong to 27 different serogroups (O106, O108, O109, O11, O123, O129, O13, O130, O140, O153, O158, O16, O173, O20, O22, O23, O28, O37, O4, O49, O55, O69, O7, O73, O74, O78 and O87, one each). All the isolates fermented lactose and glucose, were motile and urease positive. The findings of pathogenic attributes like mice pathogenicity, ST production, cytotoxigenicity in the present experiment does not correlate with any specific serogroup. Similar observations were reported earlier for various *E. coli* isolates from wild sambar (Malik *et al*. 2000).

Of the 73 isolates, 23 originated from the stud in Haryana while 50 belonged to that in UP from 40 and 67 samples collected from respective places. Depending upon the nature of faecal sample, it may be noted that normal samples, recovery rate is significantly less than those from loose or watery samples (Table 1). This indicated a role of the *E. coli* in aggravating the diarrhoeic condition of foals, even if not to be the original cause in these episodes. Moreover, the 2 studs have different rates of isolations. This may probably indicate towards a possible role of environmental conditions different in 2 farms as well as the managemental practices adopted at these places.

All isolates were resistant to 1 or more antibiotics. However, 34 foals yielding isolates had no previous history of antimicrobial exposure. It indicated that the infection might have originated from the other animals coming in contact with these young foals. Of the 23 antimicrobials used during the study, only 6 were effective on one or more cultures. Twenty one (28.8%) isolates were sensitive to ampicillin (A), 16 (21.9%) to amoxycillin (Am), 39 (53.4%) to chloramphenicol (C), 18 (24.6%) to ciprofloxacin (Cf), 13 (17.8%) to nalidixic acid (Na) and 18 (24.6%) to trimethoprim (Tr). Twenty-five isolates (34.25%) were resistant to all 23 antimicrobials. Eight isolates (10.96%) were sensitive to 5 antibiotics, 6 isolates (8.22%) to 4, 5 isolates (6.85%) to 3, 8 isolates (10.96%) to 2 and 21 isolates (28.77%) were sensitive to only 1 antibiotic. This indicated clearly to the indiscriminate use of antibiotics in animal treatment. Similar findings on multi-drug resistance of *E. coli* were reported earlier (Nemec 2008).

Cytotoxicity of cell-free culture filtrates on Vero cells indicated that 18 cultures were cytotoxigenic in nature, of these 15 were slightly cytotoxigenic and 3 were highly toxigenic causing the cells to conglomerate within 18 h and progressive destruction (Table 2). Three isolates showed vast cytopathic effects within 36 h of inoculation. However 15 others also showed the effects in about 66 h, rest 55 isolates lack cytotoxigenicity. Further the ST analysis of isolates indicated only 2 isolates, both originating from diarrhoeic foals in UP, had slight toxin production. These isolates belong to serogroup O: 6 and O: 158 respectively and showed mortality/morbidity of mice. This indicated a possible role of these toxins in the diarrhoea amongst foals. However, production of toxin is reported to be influenced by the composition of culture media, aeration and laboratory adaptation (Lallier *et al*. 1980, Klipstein *et al*. 1976). Brain-heart-infusion-broth was used in present study which may be one of the reasons for its low activity.

The pathogenicity tested by intra-peritoneal administration of cultures in mice indicated that 28 out of 73 isolates were pathogenic to mice. On post-mortem examination, congestion in lungs, sub-cutaneous tissues and intestine (enteritis) were observed. Of these 28 isolates causing morbidity, 10 had resulted in specific mortality in mice. The result of cytotoxicity and pathogenicity assays were parallel except that 9 isolates exhibiting mice pathogenicity were not cytotoxigenic; however, of these, 2 had ST activity in the culture supernatants. Only 5 isolates were producing cytopathic effects on Vero cells but were not pathogenic to mice. The magnitude of pathogenicity of different isolates in terms of mortality and morbidity varied considerably. Similar were the findings of Malik *et al*. (2000).

The results of mice mortality and verotoxicity assay indicated the pathogenic nature of about 25% *E. coli* isolates, thus emphasizing a possible role of these organisms in the episodes of diarrhoea amongst young foals and need further studies. This is significant as adult animals harbouring pathogenic organisms can serve as a source of infection to the young animals before weaning.

SUMMARY

A total of 73 isolates of *Escherichia coli* were recovered from 107 rectal/faecal swabs/faecal samples of diarrhoeic foals at organized studs in Uttar Pradesh and Haryana. The isolates belonged to 38 different serogroups with maximum number falling in O101 (10), O9 (5), O91 (4), O8 (4), O6 (3) and O156 (3). All the isolates were resistant to one or more antibiotics. The pathogenic nature of isolates was indicated by cytotoxigenicity (18 of 73 isolates) and mice pathogenicity (28 of 73 isolates) assays.

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