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Isolation, serotyping and antibiogram pattern of *E. coli* isolates associated with calf diarrhea

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Abstract
A total of 158 diarrheic fecal samples from calves were examined for identifying the etiology of the diarrhea. Out of the total samples 94 samples were found positive for *E. coli*. Serotyping of all these *E. coli* isolates revealed that all, these *E. coli* isolates belonged to 19 different serogroups and the, predominant amongst them was O2 with 14 strains, O59 with 11 strains, O123 with 10, O56 with 8 and various other strains. For the *in vitro* antibiogram, all the isolates were grouped into seven different groups and in these groups, three groups were sensitive and four resistant to Ampicillin (A), four were sensitive and three groups resistant to Ampicillin/cloxacillin (Ac), three were sensitive and four groups resistant to Chloramphenicol (C), five groups were sensitive and two groups resistant to Ciprofloxacin (Cf), six groups were sensitive and one resistant to Enrofloxacin (En), one group was sensitive and six resistant to Gentamicin (G), none of the groups was sensitive but all the seven groups were resistant to Streptomycin (S), Sulphamethiozole (SF), one group was sensitive and six resistant to Sulphadiazene (Sd) and two groups were sensitive and five resistant to Trimethoprim (Tr).

Keywords: *E. coli*, serotyping, antibiog ram, isolation, calf diarrhea

Introduction
Diarrheal disease in calves is a multifactorial health problem which despite decades of research is implicated as the major cause of morbidity and mortality in calves during the first three weeks of life, resulting in severe direct and indirect economic losses [1, 2, 3]. About 64 percent of the cases of diarrhea in calves were found related to potentially infective pathogenic agents [4, 5]. The infectious diarrhea is caused by varied etiological agents namely, bacterial, viral and parasitic. Among the bacteria *E. coli* and *Salmonella spp.* are notable while *Cryptosporidium* and *Rotaviruses* are the common protozoan and viral agents, respectively encountered in diarrhoea [6]. Therefore, the present study was conducted to study and identify the isolates, their serotypes and antibiogram pattern of *E. coli* from diarrheal samples of calves.

Materials and methods
Diarrheic fecal samples were collected from untreated neonatal calves directly from the rectum by a sterile swab. The swabs were precisely labeled and kept in the refrigerator until examined. A total of 158 fecal samples were examined during the course of study.

Media preparation and sterilization for bacterial isolation
Ready to use commercially available media marketed by Hi Media Laboratories, Private Ltd., Mumbai, 400086, India, were used in the course of present studies. Mac Conkey's Agar, Eosine Methylene Blue Agar (EMB), Nutrient Agar were used for bacterial isolation. All the media were autoclaved as per manufacturer's instructions in sterile glassware of Borosil make and their sterility of various media was ascertained by incubation overnight at 37 °C.

Isolation of *E. coli* isolates
Isolation of *E. coli* isolates and standardized procedures to perform biochemical tests were done as per the method described by Crichton [7] and WHO [8]. Fecal samples were transported to the laboratory in ice and were processed within 2h of collection. Mac- Conkey's Agar and Eosine Methylene Blue Agar (EMB) were used for isolation of *Escherichia coli*. A loop full of fecal material was streaked on Mac Conkey's Agar and was incubated overnight at 37 °C. Following incubation, characteristic pink colonies suspected for *E. coli* were picked up and sub-cultured on Eosine Methylene Blue Agar (EMB) and incubated overnight at 37 °C.

Colonies with characteristic metallic sheen were considered as pure growth. *E. coli* isolates which were identified on the basis of cultural characteristics and subjected to biochemical confirmation. The representative *E. coli* colonies on nutrient agar slants were sealed with paraffin film and stored at 4°C for further use.

Serotyping of *Escherichia coli*

All the *E. coli* isolates identified on the basis of morphology, cultural and biochemical characteristics were sent to Central Research Institute, Kasauli (H.P) for serological typing.

Antibacterial sensitivity testing

Disc diffusion method was used for antibacterial sensitivity testing [9]. Antibiotic discs were obtained from Hi - Media Laboratories, Bombay. The isolates were designated as Resistant (R) and Sensitive (S) to various antibiotics as per zone diameter [10]. Following antibiotic discs were used:

Antibacterials	Disc Content (in µg)
Ampicillin (A)	10
Ampicillin/cloxacillin (Ac)	10
Chloramphenicol (C)	50
Ciprofloxacin (Cf)	30
Enrofloxacin (En)	10
Ofloxacin (Of)	01
Sulphadiazene (Sd)	300
Sulphamethiozole (Sf)	300
Streptomycin(S)	10
Trimethoprim(Tr)	10

Results and discussion

Etiology of calf diarrhea

Etiological agents

Spectra of etiological agents identified in the present study are presented in Table 1. Non-infectious scours accounted for 29.7 per cent incidence. Infectious scours category included pathogens of varied spectra *viz*., bacterial, viral, protozoa and helminths. Bacterial agents identified were *E. coli, Salmonella sp., Clostridia sp.* Amongst *E. coli* per cent incidence was recorded with one case (0.6) of enter pathogenic *E. coli* infection and three cases (1.9) with shiga toxin producing *E. coli* infection remaining serotypes (90) accounted for 48.7 per cent morbidity. *Salmonella sp., and Clostridia sp.,* accounted for 1.9 and 2.5 per cent morbidity. Viral etiology was represented by *Rotavirus* contributing to 3.8 per cent morbidity. Amongst the protozoan scours, *Eimeria* was the single etiological agent with morbidity of 4.4 per cent. Helminth scours included *Strongyloides, Amphistome* with a morbidity per cent of 3.8 and 1.3, respectively. Our findings are in concurrence with the facts recorded by Clement [11]. Similar to our study, many workers have frequently isolated an assortment of infectious agents from calves with diarrhea, which included several types of enter pathogenic viruses, bacteria and protozoa in various combinations as revealed in the present study [12, 13, 14, 15].

Table 1: Etiology of calf diarrhea as observed in the present study

S. No.	Etiology	Morbidity (No.)	Per cent incidence
1	Non-infectious/Nutritional scours	47	29.7
2	Infectious scours		
A	Bacterial scours		0.0
I	Enteropathogenic *E. coli*	1	0.6
II	Shiga toxin producing *E. coli*	3	1.9
III	Others (90 *E. coli* isolates)	77	48.7
Iv	*Salmonella. Spp.*	3	1.9
V	*Clostridia spp.*	4	2.5
B	Viral Scours		0.0
I	*Rotavirus*	6	3.8
C	Protozoan scours		0.0
I	*Eimeria spp.*	7	4.4
D	Endoparastic scours		0.0
I	*Strongyloides*	7	4.4
II	*Amphistome*	3	1.9
	Total	158	

Serogrouping of *E. coli* isolates

The various serogroups identified from diarrheal calves and their respective strains have been indicated in Table 2. In all 94 *E. coli* isolates from 158 diarrheal samples were isolated and sent for serotyping to Central Research Institute, Kasauli (H.P).

Table 2: Number and Percentage of 'O' serogroups of *E. coli i*solated from diarrheal samples

S. No	Somatic Group	No. of Strains	Percentage of serogroups
1	O148	2	2.13%
2	O101	1	1.06%
3	O3	1	1.06%
4	O41	1	1.06%

5	O68	1	1.06%
6	O111	1	1.06%
7	Rough(R)	1	1.06%
8	O5	3	3.19%
9	O36	4	4.26%
10	O8	5	5.32%
11	O60	5	5.32%
12	O80	5	5.32%
13	O84	6	6.38%
14	O9	6	6.38%
15	O56	8	8.51%
16	UT	9	9.57%
17	O123	10	10.64%
18	O59	11	11.70%
19	O2	14	14.89%

Quantification of serogroups

Number and percentage of O serogroups of *E. coli* isolated from diarrheal calves has been shown in Table 2. In all, *E. coli* isolates belonged to 19 different serogroups. However, the predominant amongst them was O2 (14.89 per cent) with 14 strains followed by O59 with 11 (11.70 per cent) strains, O123 with 10 (10.64 per cent), UT with 9 (9.57 per cent), O56 with 8 (8.51 per cent), O9 with 6 (6.38 per cent), O84 with 6 (6.38 per cent) O80 with 5 (5.32 per cent), O60 with 5 (5.32 per cent), O8 with 5 (5.32 per cent), O36 with 4 (4.26 per cent), O5 with 3 (3.19 per cent), O148 with 2(2.13 per cent) strains and Rough, O111,O68,O41, O3, O101 with one strain each (1.06 per cent). In the present study, prevalence of large number of serogroups indicate a wide spread presence of pathogens and non-pathogens / commensal in neonatal diarrheal calf herds of Jammu region. In all 94, *E. coli* isolates from 158 diarrheal samples belonging to 19 serogroups were isolated in the present study. Our study on diarrhoeic serotypes is consistent with the findings of many workers [16, 17, 18].

Pathogenic *E. coli* groups

Pathogenic *E. coli* have been represented in Table 3. Two pathotypes of *E. coli* were isolated in the presented study. One serogroups of EPEC and three serogroups of STEC were isolated from 94 *E. coli* isolates. One serogroups of EPEC (O111) and three serogroups of STEC were isolated from 94 *E. coli* isolates. The pathogenicity of most typical EPEC serotypes has been confirmed by studies of Nataro and Kaper [19] and EPEC strains are defined as intimin-containing diarrhoeagenic *E. coli* that possess the ability to form "attaching and effacing" lesions on intestinal cells depicting them as important diarrheal pathogen.

Table 3: Pathogenic *E. coli* isolated in diarrheal calves

S. No.	Somatic group	No. of strains	Path Types
1	O111	1	EPEC
2	O2	1	STEC
3	O148	1	STEC
4	O84	1	STEC

***In vitro* sensitivity and resistant pattern of *E. coli* isolates**

The relative response of *E. coli* serogroups to different antimicrobials *in vitro* is recorded in Table 4. In all, 10 antimicrobials were tested against *E. coli* serogroups and the results were recorded as Resistant (R) and Susceptible (S). The antimicrobials tested for *E. coli* serogroups were Ampicillin (A), Ampicillin/cloxacillin (Ac), Chloramphenicol (C), Ciprofloxacin (Cf), Enrofloxacin (En), Gentamicin (G), Streptomycin (S), Sulphadiazene (Sd), Sulphamethiozole (Sf) and Trimethoprim (Tr). 94 serogroups isolated in the present study were grouped under seven groups *viz*., G1 with 19 serogroups, G2 with12 serogroups, G3 with 19 serogroups, G4 with 18 serogroups, G5 with18 serogroups, G6 with 7 serogroups and G7 with 1 serogroup as per their sensitivity and resistance pattern. Three groups were sensitive and four resistant to Ampicillin (A), four were sensitive and three groups resistant to Ampicillin/cloxacillin (Ac), three were sensitive and four groups resistant to Chloramphenicol (C), five groups were sensitive and two groups resistant to Ciprofloxacin (Cf), six groups were sensitive and one resistant to Enrofloxacin (En), one group was sensitive and six resistant to Gentamicin (G), none of the groups was sensitive but all the seven groups were resistant to Streptomycin (S), one group was sensitive and six resistant to Sulphadiazene (Sd), none of the groups was sensitive but all the seven groups were resistant to Sulphamethiozole (Sf) and two groups were sensitive and five resistant to Trimethoprim (Tr).

Previous findings by Tikoo [20] on resistance pattern of diarrheal *E. coli* of farms of Jammu are in concurrence with our findings. Resistance to various groups of antimicrobials of this organism has been reported by a number of workers, which is suggestive of judicious use of antimicrobials in diarrheal syndrome [21, 22, 23]. Indiscriminate use of antibiotics is the main factor resulting in this emergence, selection and dissemination of drug-resistant pathogens in both veterinary and human medicine, posing a challenge to clinicians [23].

Table 4: *In vitro* sensitivity pattern of *E. coli* serogroups

S. No.	Group (No. of isolates)	Serogroup (number of strains)	Name of Antimicrobials									
			A	Ac	C	CF	En	G	S	SD	SF	TR
1	G1(19)	O59 (3), O8 (2), O3 (1), O41 (1), O84 (3), O80 (2), O56 (1), O111 (1), O123 (3), O9 (1), R (1).	S	S	S	S	S	R	R	R	R	R
2	G2(12)	O59 (2), O9 (1), O5 (1), O68 (1), O84 (2), O2 (3), UT (2).	R	S	S	S	S	R	R	R	R	S
3	G3(19)	O9 (3), O123 (3), O56 (3), O84 (1), O2 (2), O60 (2), O101 (1), O8 (1), O36 (1), O148 (2).	S	S	S	S	S	S	R	R	R	S
4	G4(18)	O59 (3), O2 (6), O36 (3), O56 (3), O80 (3).	S	S	R	S	S	R	R	S	R	R
5	G5(18)	O59 (3), O8 (2), O60 (3), O2 (3), O56 (1), O123 (2), O5 (1), UT (3).	R	R	R	S	S	R	R	R	R	R
6	G6(7)	O123 (2), O5 (1), UT (4).	R	R	R	R	S	R	R	R	R	R
7	G7(1)	O9 (1)	R	R	R	R	R	R	R	R	R	R

*(A) Ampicillin 10µg, (Ac) Ampicillin/cloxacillin10 µg, (C)Chloramphenicol 50 µg, (Cf)Ciprofloxacin 30µg (En)Enrofloxacin 10µg, (G)Gentamicin 50µg, (Sd) Sulphadiazene 300µg, (Sf) Sulphamethiozole 300µg, (S) Streptomycin 10µg, (Tr) Trimethoprim 10µg

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