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Prevalence of *E. coli* Serogroups Associated with *Neonatal* Calf Diarrhea

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ABSTRACT

The aim of the present study was to determine the prevalence of serogroups of *Escherichia coli* isolated from diarrheic calves in the Bikaner district of Rajasthan. All the 35 isolates had been serogrouped from National Salmonella and *Escherichia coli* centre, Kasauli, H.P. (India). The isolates belonged to O17, O18, O20, O111, O118, O120, O121, O128 and Untypable serogroups and most prevalent serogroups being O18 and O121 with 20%. In conclusion, this study supplemented the information about prevalence of different serotypes of *E. coli* in Bikaner. In the present study O118, O120, O121 and O128 serogroups were identified which belongs to STEC pathotypes which have public health significations.

HIGHLIGHTS

- Most prevalent serogroups was being O18 and O121.
- Serogroups O118, O120, O121 and O128 were identified which have public health significations.

Keywords: Serogroups, *Escherichia coli,* diarrheic calves, STEC, ETEC.

Neonatal diarrhea is amongst the most common illnesses influencing calves, leading to reduction in milk production and calf growth that affects dairy sector economies. Although *Escherichia coli* is a commensal microorganism in the gastrointestinal tract, some serotypes are known to cause high prevalence of diarrhea and food poisoning.

A method based on the identification of the combination of three principal cell surface components, the O-antigens, flagellar H-antigens, and capsular K-antigens was developed for subtyping *E. coli* strains. Since few laboratories had capabilities to type K-antigens, serotyping based on O- and H-antigens became the "gold standard" for *E. coli* typing. Currently, 186 somatic (O), 55 flagellar (H) and 80 capsular (K) antigens of *E. coli* have been identified, and there are over 160 serological types of *E. coli* (Fratamico *et al*., 2016; Sarowska *et al*., 2019). O-groups numbered O1-O188 have been defined, except for O31, O47, O67, O72, O94, and O122 that have not been designated and four groups have been divided into subtypes O18ab/ac, O28ab/ac, O112ab/ac, and O125ab/ac, giving a total of 186 O-groups (Debroy *et al*., 2016; Navarro *et al*., 2021). Serology has defined 53 H-flagellar antigens that are numbered from H1 to H56, but H-types 13, 22, and 50 are not in use (Fratamico *et al*., 2016; Mare *et al*., 2022).

Several investigations have been conducted to study the prevalence of *E. coli* serotypes in different parts of India. However, limited studies regarding identifying *E. coli* serotypes in diarrheic calves from Bikaner district of Rajasthan have been conducted till date and findings of this research may add to the available data.

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MATERIAL AND METHODS

In this study, 35 *E. coli* isolates were selected for the serogrouping study. All these isolates were isolated from diarrheic calves below one month's age and confirmed by culture, biochemical and 16s rRNA species-specific based ribotyping (Kumar *et al.,* 2022).

Fig. 1: Geographical representation of Bikaner district of Rajasthan, India

Serogrouping of the *E. coli* Isolates

The isolated samples which were confirmed as *E. coli* by cultural, Gram's staining, biochemical procedures and molecular detection, were submitted to National *Salmonella* and *Escherichia* Centre, Central research institute, Kasauli, Himachal Pradesh on nutrient agar stab cultures, for serogrouping. Briefly, test strain was inoculated into 5mL nutrient broth and incubated at 37°C for overnight with agitation. Bacterial growth was boiled at 100°C for one hour, and then formalin was added to a final concentration of 0.3% (Test antigen). For testing with pooled sera, 50 µL of 16 pools of O antisera was added to 96-wellplate. Then 50 µL of test antigen was added to each well. A negative control well was set up with 50 µL each of antigen and saline, respectively. Plates were incubated at 37°C overnight and observed for agglutination reaction. Test strain showing agglutination in all wells including negative control, strain was regarded "rough." If agglutination was seen with single pool, then next agglutination test was set up with factor sera constituting the pool. But if agglutination was seen with more than one pool, then antigen was titrated against all sera constituting the pools. The test antigen which even did not show agglutination following antigen preparation at 121°C for 2½ hours was regarded as "untypeable."

RESULTS AND DISCUSSION

Escherichia coli serogrouping is used as a conventional method for pathogen characterization and diagnosis. Besides, the potential use of O antigen characterization, *E. coli* associations with O antigens varies across different geographical regions. A shift in O serogroups along with their virulence attributes has been observed (Sarkar *et al.,* 2014). The significance of serotypes of *E. coli* and role of structural components of a bacterial cell are essential attributes to determine the pathogenesis. As *E. coli* pathotypes possess a large number of different "O" somatic antigens; therefore, their continuous monitoring is helpful in subtyping strains and enhancing phylogenetic studies (Thakur *et al.,* 2018).

In this study, all 35 *E. coli* isolates belonged to eight different serogroups and the most predominant serogroups were O18 and O121 with seven isolates each followed by O111 and O120 with four isolates each (Table 1). Moreover, there were seven (20%) isolates which were Untypable with the available antisera. In the present study, the prevalence of different serogroups of *E. coli* indicated a widespread presence of pathogens and non-pathogens / commensals in diarrheic calves in the Bikaner district of Rajasthan.

The frequencies of the typable strains vary depending on two factors. The first one is the availability of different O serogroup-specific antisera with the typing center and the second prevalence of common serotypes among the isolates. The somatic group O17 reported in this study has been previously reported by Pachaury *et al.* (2013) and Bhat *et al.* (2017) from diarrheic calves. Untypable and Rough types have also been reported by Wani *et al.* (2013) from diarrheic calves. In the present study, O18, O20 serogroups were detected which are identified as enterotoxigenic *Escherichia coli.* The serogroup O20 has been also previously reported by Sharma *et al.* (2017) and Bhat *et al*. (2017) from diarrheic calves. The serogroup

Table 1: Prevalence of *E. coli* serogroups

Sl. No.	Serogroups	Isolate ID	Total no. of *E. coli* isolates	Prevalence of serogroups (%)
1	O17	S33	1	2.85
2	O18	S11, S15, S16, S24, S35, S37, S39	7	20
3	O20	S36	1	2.85
4	O111	S1, S7, S17, S29	4	11.42
5	O118	S30	1	2.85
6	O120	S4, S12, S21, S23	4	11.42
7	O121	S5, S8, S9, S22, S26, S31, S34	7	20
8	O128	S14	1	2.85
9	Untypable (U.T.)	S2, S3, S13, S20, S25, S32, S38	7	20
10	Rough	S18, S28	2	5.71

O20 was characterized as diarrheagenic enterotoxigenic *E. coli* by Thakur *et al.* (2018).

The serogroup O111 was characterized as EAEC, EHEC and EPEC with different associated H antigens. The serogroup O111 was mainly reported as EHEC and EPEC strain by Galal *et al*. (2013), Shahrani *et al*. (2014), Khalifa *et al*. (2019), Algammal *et al.* (2020) and Tikoo *et al*. (2021). EHEC strains of the O157:H7 serotype are the most important EHEC pathogens in North America, the United Kingdom and Japan, but several other serotypes, particularly those of the O26 and O111 serogroups, can also cause disease and are more prominent than O157:H7 in many countries. Several serotypes (e.g., O26:H11, O113:H21, O26, and O111) of STEC are zoonotic in nature (Torres *et al.*, 2018).

El-Seedy *et al.* (2016) reported serogroups O111 with ETEC virulent gene (*K99*) in neonatal diarrheic calves. The serogroup O118 was detected previously by Khalifa *et al*. (2019) in diarrheic calves. Shikha *et al*. (2021) reported serogroup O118 as EPEC strains from sheep. Srivani *et al.* (2020) reported that serogroups of STEC associated with calf diarrhea include O120, O121 and O128 which run hand to hand with present findings. Shahrani *et al*. (2014) also reported serogroups O121 and O128 but O26 and O157 were the most common serogroups in diarrheic calves from Iran. Algammal *et al.* (2020) observed O128 as the most common serogroups in diarrheic calves. The 12 serogroups originally recognized by the World Health Organization as EPEC or the classical EPEC (Shikha *et al*., 2021), out of two serogroups O111 and O118 were found in our study.

Abed and Menshawy (2019) reported serogroup O26 as the most prevalent (21.1%) followed by O103 (18.4%) then, serogroups O86 and O111 (13.2% and 10.5%, respectively) from neonatal calf diarrhea in Egypt. Moreover, other serogroups were recorded; O119, O127 and O157 (5.3% for each) and O18, O44 and O158 (2.6% for each) while, 13.2% of *E. coli* isolates were untyped. Moussa and Shama (2020) recovered seven *E. coli* serotypes (O26, O157, O78, O125, O146, O44, and O18), and O26 and O157 were the most common from calves in Egypt.

The highest percentage was found for O20 serotype (22.48%) followed by O22 (14.73%) and O69 (10.08%) from diarrheic calves in Kashmir valley (Bhat *et al*., 2017) while Manzoor *et al*. (2015) had reported O15 and O26 as predominant serotypes in calves from Kashmir valley.
Escherichia coli strains are invasive and belong to certain serogroups with virulence properties (Denamur *et al.,* 2021). The relative importance of individual serogroups seems to vary between countries. It has been established that typeable isolates from colisepticemia can belong to a relatively small number of O serogroups (Papouskova *et al.,* 2020). The distribution of different serotypes of *E. coli* varies with geographical regions and their prevalence in man and animals in a particular area.

CONCLUSION

In conclusion, this study provided the information about prevalence of different serogroups of *E. coli* in Bikaner. In the present study, O118, O120, O121 and O128 serogroups were identified which are associated with

STEC pathotypes which have public health significance. The most prevalent serogroups were O18 and O121. Cattle may be important reservoirs of these serogroups and are an important source of environmental contamination. Further, data based on O serogroups identified in this study may be useful in preparation of area specific vaccines to control colibacillosis in calves and thus reduce the use of antibiotics in future.

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ETHICS APPROVAL

This study was conducted following approval by the research committee and Institutional Animal Ethics Committee (Reg. No.-2044/GO/Re/SL/18/CPCSEA), Rajasthan University of Veterinary and Animal Sciences, under permission number CVAS/IAEC/2021-22/108.

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