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Occurrence of *E. coli* and associated risk factors in neonatal calf diarrhea from Bikaner

Sudesh Kumar, Suvidhi, Kritika Dhial, Mahender Milind and Taruna Bhati

Abstract
Present study investigated occurrence of *Escherichia coli* and factors associated with calf diarrhea under field condition. In the study, 39 fecal samples were collected from diarrheic calves below one month of age with the record of breed, age and sex variables. All the *E. coli* isolates produced pink colonies on MacConkey agar, green metallic sheen on EMB agar with characteristic IMViC pattern (+ + - -), out of which 35 *E. coli* isolates produced an amplicon of 662 bp size by 16S rRNA ribotyping with overall prevalence being 89.74% (35/39). Occurrence of *E. coli* in Holstein-Friesian, male calves, age group-I (1-10 days), unorganized farm and small herd size was 100%. In conclusion, the present study suggests the importance of maintenance of strict hygienic measures to prevent diarrhea in the neonatal calves.

Keywords: Calf diarrhea, calf management *Escherichia coli*, hygiene, risk factors

Introduction
Neonatal calf diarrhea (NCD) is a major disease globally when calves are reared intensively, and constitute substantial cost in terms of calf mortality, opportunity costs for labor and capital, costs of veterinary and loss in calf value. It is a multi-factorial disease where, besides the causative pathogenic agent, calf age, management and environmental factors, may influence the clinical outcome of disease (Bruning-Fann *et al.*, 1992) [5].
Neonatal calf diarrhea, defined as diarrhea occurring during the first month of the life, is the most common disease and the cause of death of calves worldwide in this period of life. *Escherichia coli*, *Bovine rotavirus*, *Bovine coronavirus*, *Salmonella* spp., *Cryptosporidium parvum* are common etiological agents of NCD. These pathogens can cause disease as mono or co-infections and also may be found in clinically healthy animals (Umpierrez *et al.*, 2016) [29].
Escherichia coli (*E. coli*) is Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobe and is classified under the family Enterobacteriaceae. Calves are vulnerable to *E. coli* infection. First week age groups appear to be susceptible more. Symptoms include diarrhea, a rise in temperature, weakness and lack of appetite.
Bikaner has a high cattle population and occurrence of colibacillosis is on the higher side (76%) in Bikaner (Sharma *et al*, 2017) [25]. Additionally, the climate of Bikaner has been changed in the last few decades (Chaudhary *et al.*, 2009) [7] due to increased irrigation facilities and global climate changes. The livestock sector in Bikaner plays an important role in the farmer economy with the direct and indirect contribution in agriculture. The present study was carried out to evaluate the occurrence of *E. coli* in cases of diarrhea in young calves in Bikaner from January 2021 to March 2022.

Materials and Methods
Collection of fecal samples
The study was conducted in Bikaner region of Rajasthan located at western part of India where climate is arid. A total of 39 fecal samples were collected from calves below one month of age showing classical clinical signs of diarrhea at private dairy farms as well as animals of individual holdings in the Bikaner district of Rajasthan. The samples were collected aseptically in sterile containers and transported to lab for further processing as soon as possible.

Processing of the samples
The sample swabs were directly streaked over selective MacConkey agar followed by

incubation at 37 °C for 24 h. The isolation and identification of *E. coli* was carried out as per Edward and Ewing (1986) and Quinn *et al.* (2005) [11, 21]. The organisms were confirmed on the basis of cultural characteristics and biochemical test profiles. Further molecular confirmation was done using 16S-rRNA ribotyping as per Khaled *et al*. (2010) [13].

Results

Occurrence of *Escherichia coli* in diarrheic calves

In the present study, out of 39 faecal samples collected from diarrheic calves, 35 (89.74%) samples were found positive for *E. coli* by conventional and 16S rRNA ribotyping methods.

The highest percentage of *E. coli* positive samples was detected in age group-I (1-10 days), *i.e.*100% followed by 66.66% in age group-II (11-20 days) and 60% in age group-III (21-30 days). Breed-wise, a higher *E. coli* isolation rate (100%) was recorded in Holstein Friesian calves followed by 86.67% in indigenous (Rathi) breed calves. All male calves (100%) yielded a higher *E. coli* isolation rate as compared to female calves (85.18%) (Table1).

Table 1: Occurrence of *E. coli* based on age, breeds, and sex of the calves

S. No.	Variable	Category	Total No. of samples	No. of positive samples	Occurrence of *E. coli* (%)
1.	Age	I (0-10 days)	28	28	100
2.		II (11-20 days)	6	4	66.66
3.		III (21-30 days)	5	3	60.00
4.	Breed	Holstein Friesian	9	9	100
5.		Indigenous (Rathi)	30	26	86.67
6.	Sex	Male	12	12	100
7.		Female	27	23	85.18
8.	Farming Practices	Organized	19	16	84.21
9.		Unorganized	20	19	95.00
10.	Herd Size	>10 Bovines	19	16	84.21
11.		6-10 Bovines	11	10	90.90
12.		1-5 Bovines	9	9	100
13.	All over	Total	39	35	89.74

Discussion

Occurrence of *Escherichia coli* in diarrheic calves

The overall occurrence of *E. coli* isolates in diarrheic calves was found to be 89.74% (35/39) in the Bikaner district of Rajasthan. Nayak *et al.* (2019) [17] and Rathor *et al.* (2021) [23] reported similar results with 79.41% (81/102) and 88% (22/25) occurrence of *E. coli* from diarrheic calves from the same area of study. A higher prevalence of *E. coli* in diarrheic calves fecal samples was also observed by other workers from different states in India *viz*. 71.65% by Wani *et al.* (2013) [31] in Kashmir, 88% by Diwakar and Diwakar (2015) [9] in Uttar Pradesh, 72.58% by Malaviya *et al.* (2017) [14] in South Gujarat, 61.9% by Batabyal *et al.* (2020) [4] in West Bengal and also worldwide *viz.* 86.67% by Pourtaghi *et al*. (2013) [19] in Iran and 73.26% by Bakry *et al.* (2020) [3] in Egypt. On the other hand, a lower prevalence of 36.66% in TamilNadu and 46.51% in Andhra Pradesh and Telangana States was reported by Manickam and Ponnusamy (2017) [15] and Srivani *et al.* (2017) [26] respectively from India and 27% by Dawod *et al.* (2016) [8] and 28.83% by Algamma *et al.* (2020) [2] in Egypt. The high prevalence of *E. coli* from cases of calf diarrhea in the present study could be attributed to the variations in environmental and management conditions of the farms such as insufficient and/or poor quality colostrum feeding/intake, gaps in management specifically calf handling practices including inadequate nutrition, exposure to the harsh environmental conditions, insufficient attention to the newborn calf, or a combination of these (Charles *et al.*, 2003; Radostits *et al.*, 2007) [6, 22]. In addition to that, poor hygiene often allows buildup of pathogenic strains in the young animal's environment and a large dose of pathogenic *E. coli* may overcome colostral immunity (Quinn *et al.*, 2005) [21].

In the present study, the association between different age groups, breeds and sex with the occurrence of diarrhea due to *E. coli* was studied. The isolation rate of *E. coli* from diarrheic feacal samples decreased with increasing age of calves. The earlier studies also support the present observation that clinically, age group-I (1-10 days) calves were most commonly found to be suffering from colibacillosis (Shahrani *et al*., 2014; Abdulgayeid *et al*., 2015; Dawod *et al*., 2016; Srivani *et al*., 2017; Nayak *et al.*, 2019) [24, 1, 8, 26, 17]. Neonatal calves during first week of life are particularly more susceptible because the normal intestinal flora is not yet fully developed; they have a naive immune system and also have receptors for the adhesions of *E. coli* (Villarroel, 2009 and Patel *et al.*, 2019) [30, 18]. Also, *E. coli* can colonize the sterile intestine at the early stage of neonate and those newborn calves are more susceptible than adults especially if they take low colostrum (Quinn *et al*., 2005) [21]. The cause of high isolation rate of *E. coli* in age group-I (1-10 days) calves receiving less amount of colostrum could be for various reasons *i.e*. supply of colostrum of inadequate quality and quantity and delay in first colostrum feeding, which leads to failure of transfer of passive immunity (FPT). Calves with inadequate colostral immunoglobulin concentration within 24 h of birth were found at greater risk of neonatal morbidity and mortality. In addition, FPT could be due to bacterial contamination of the fed colostrum (Keith *et al.*, 2010 and Meganck *et al.*, 2014) [12, 16].

A higher *E. coli* isolation rate (100%) was recorded in Holstein Friesian calves followed by 86.67% in indigenous calves (Rathi). Rathi breed of cattle is a gift of nature to the arid north-west part of Rajasthan. While being reared under extremely harsh conditions, the animals of this breed have developed extreme tolerance against environmental variations and high resistance against various diseases (Purohit and Sharma, 2001) [19].

Male animals generally receive less attention and managed care as their role in the farm is considered irrelevant especially as the replacement stock (Gebregiorgis *et al.*, 2016) [11] which explains that all male calves in our study were positive for *E. coli*. Similar study by Tedla and Degefa (2017) [28] showed a prevalence of 11.11% and 7.99% in male and female calves, respectively. A higher prevalence of *E. coli* (51.6%) infection was observed in male calves, followed by female calves (41.0%) by Tadesse (2020) [27] in Ethiopia.

However, sex failed to show significant difference (P > 0.05) with the prevalence of *E. coli*.

It is important to isolate any calves showing signs of scours and use strict hygiene principles to keep them from spreading any pathogens to the healthy calves. The load of pathogens in the calf farms can be reduced through improving host immunity. Care takers should understand what the hazards are and why protocols for cleaning and disinfection are needed. Reduction of pathogen load in the calf environment will reduce the burden of illness on the calves, as well as reduce the potential for pathogens to reach the human population. In conclusion, the present study suggests the importance of maintenance of strict hygienic measures to prevent diarrhea in the neonatal calves.

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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.

Competing interests

Authors have declared that no competing interests exist.

Authors' contributions

This work was carried out in collaboration among all authors. Author S.K. designed the study, wrote theprotocol, conducted the experiments and wrote the first draft of the manuscript. Authors …….. managed the analyses of the study and contributed in conducting experiments. Author T.B. supervised all the authors for conducting this research, guided in methodology and analysis of the study and also reviewed the original draft. All authors read and approved the final manuscript.

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