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A study of microbial evaluation of calf diarrhea in the geographical area of Bikaner

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Abstract
The objective of the present study was to identify the various bacterial causal agents (*Salmonella* spp. and *E. Coli*) in cattle calves that had diarrhea. Irrespective of the breed or age, cattle calves below 3 months of age exhibiting the typical clinical symptoms of diarrhea were presented at the Veterinary Clinical Complex, College of Veterinary and Animal Science, RAJUVAS, as well as in the nearby local region of Bikaner. A total of 45 diarrheal calves that were screened negative for endoparasite infestation and next subjected to a microbiological and cultural examination. Hi Enterobacteriaceae Identification kit (KB003) was used for identification following bacteriological culture and isolation. 34 of the 45 faecal samples were positive for a single isolate, meaning that 26 (57.77%) of the samples contained Escherichia coli and 8 (17.78%) had *Salmonella* spp. The other 11 (24.44%) faecal samples tested positive for a mixed *Salmonella* spp. and *E. Coli* infection. The research concludes that the most often isolated causal bacteria for calf diarrhea were *E. Coli* following *Salmonella* spp.

Keywords: Bikaner, microbial, evaluation, geographical, various

Introduction
Calf diarrhea is one of the most common intestinal infections in young animals and causes significant financial losses for the dairy industry worldwide due to lost output and offspring (Cho and Yoon, 2014) [7]. According to Azizizzadeh *et al*. (2012) [1], diarrhea in calves is most common in the first week of life and is associated to electrolyte and water loss, which can result in hypotonic or isotonic dehydration. According to Ramkumar (2012) [19], the respiration rate, pulse, and rectal temperature were all within normal ranges. Most enteropathogens associated with diarrhea in calves have been identified as *Salmonella*, *Clostridium*, and *E. Coli* species (Singla *et al*., 2013; Cho, and Yoon, 2014) [21, 7]. The pathogens Escherichia coli, *Salmonella* spp., rotavirus, coronavirus, and *Cryptosporidium* spp. were the primary objectives of investigations into calf diarrhea; however, more recent research has indicated that co-infection could have a significant role in the pathogenesis of gastrointestinal disorders (Gomez and Weese, 2017) [9]. *E. Coli* was successfully isolated through EMB, MaCconkey agar, and selective media.

Materials and Methods
Source of animals
Irrespective of the breed or age, cattle calves below 3 months of age exhibiting the typical clinical symptoms of diarrhea were presented at the Veterinary Clinical Complex, College of Veterinary and Animal Science, RAJUVAS, as well as in the nearby local region of Bikaner. A total of 45 diarrheal calves that were screened negative for endoparasite infestation and next subjected to a microbiological and cultural examination.

Clinical observations
According to Radostitis *et al*. (2007) [18], a thorough assessment of each calf's clinical measures, such as physical condition, rectal temperature, pulse rate, respiration rate, appetite, frequency of defaection, color and consistency of faeces, depression, and dehydration, were carried out.

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Collection of faecal samples

As per the protocol by Hansen and Perry (1990), feces samples were taken via rectum from calves that had diarrhea and kept in polythene bags in the refrigerator. Samples of feces were collected for the purpose to research gastrointestinal parasites. Additionally, feces samples have been collected with the objective to cultivate and identify the bacteria associated with calf diarrhea. Samples of feces were collected from apparently healthy and diarrheal calves and examined in a lab.

Isolation and identification of bacteria

Faecal sample cultural examination was carried out to isolate bacteria in accordance with Cowan and Steel's (1975) standard procedure, and biochemical kit identification was used for identification.

The high culture sterile swab implemented to collect the feces samples. The primary, secondary, and tertiary streaking of each faecal sample onto nutrient agar plates produced isolated bacterial colonies. These petri plates were incubated at 37°C for 24 hr. These isolated colonies were incubated for 24 hours, and then they were sub cultured on XLD agar and Eosin Methylene Blue Agar (EMB) to isolate *Salmonella* spp. and Escherichia coli and incubated for 24 hrs and 48 hrs for *Escherichia coli* and *Salmonella* spp., respectively. The confirmation of the isolates such as *Escherichia coli* and *Salmonella* spp. was done by using biochemical test kits (HiEnterobacteriaceae identification kit KB003).
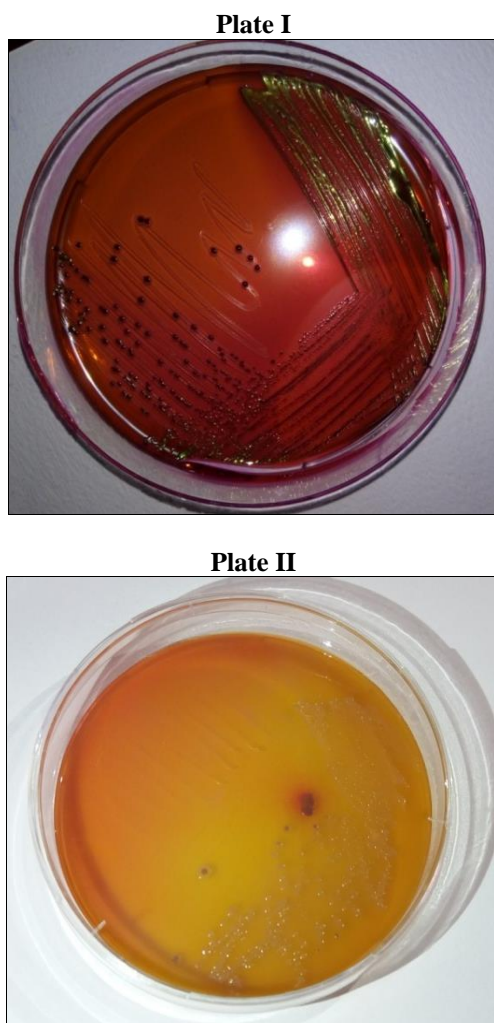
Plate I

Plate II

Fig 1: Plate A demonstrates *E. Coli* colonies on EMB agar with a green metallic sheen while Plate B demonstrates *Salmonella* colonies on XLD agar with a black head.

Fig 2: Plate III demonstrates *E. Coli* on KB003 Hi25 Enterobacteriaceae while Plate IV shows Identification Kit *Salmonella* on KB003 Hi25 Enterobacteriaceae Identification Kit

Results and Discussion

Faecal samples of all 45 diarrhoeic calves which were found negative for endoparasites by faecal examination were investigated for microbiological cultural examination. In the present study, after bacteriological culture and isolation; the identification was done by HiEnterobacteriaceae Identification kit (KB003). The results of microbial examined are presented in Table 1. 34 of the 45 faecal samples were positive for a single isolate, meaning that 26 (57.77%) of the samples contained Escherichia coli and 8 (17.78%) had *Salmonella* spp. The other 11 (24.44%) faecal samples tested positive for a mixed *Salmonella* spp. and *E. Coli* infection. In the present study, the percentages of different bacterial isolates in the faecal samples of diarrhoeic calves are presented in Table 2. *E. Coli* was found in 32 (71.11%) samples and *Salmonella* spp. in 13 (28.89%) samples. *E. Coli* was the most often isolated causal bacteria in the current investigation, followed by *Salmonella* spp. Similar to present findings Sharma (2013) [20] reported *E. Coli* as single isolate in 76% samples and mixed bacterial pathogen isolates (*E. Coli* + *Salmonella* spp.) in 24% faecal samples of calf diarrhoea. Gay, CC and Besser, TE (1994) [12] correspondingly found similar outcomes, identifying *E. Coli* as the most frequent cause of diarrhea in calves. Worldwide, 75–95% of cases of newborn calf diarrhea are caused by bacteria such as *E. Coli*, *Salmonella* spp., rotavirus, and *Cryptosporidium* spp. (Hansa *et al.*, 2012) [14]. *Salmonella* spp. were observed to be less common and *E. Coli* to be more common among other workers. When calves had diarrhea, Aydin *et al.* (2001) [2] discovered that 92.07% of cases tested positive for *E. Coli* and 0.99% for *Salmonella* spp. According to Chaurasia (2001) [6], 87% of the faecal samples from calves that had diarrhea included *E. Coli*. According to Sharma (2013) [20], feces samples from diarrheal cattle calves included 86% *E. Coli*. and 3% *Salmonella* spp. 62.36% of diarrheal samples tested positive for *E. Coli*, while 4.30% tested positive for *Salmonella* spp., according to Arora *et al.* (2010) [3]. According to Ghanem *et al.* (2012) [10], 26.70% of the

faecal samples from calves who had a diarrheal problem showed *Salmonella* spp. and 73.30% contained an isolate of *E. Coli*. *E. Coli* was found in 25% of samples and *Salmonella* in 8.8% of samples, according to Ansari *et al*. (2014) [4]. In cases of diarrheal newborn calves, *E. Coli* was isolated from 31.81% and 72.22% of the faecal samples by Asati *et al*. (2008) [5] and Kumar *et al*. (2010) [15], respectively. In 80% of the cases of diarrhea in calves, Nayak *et al*. (2019) [17] also isolated *E. Coli* through culture examination, which was confirmed by PCR.

Various in other research Of the 286 cow calves that were examined, 122 (42.65%) tested positive for calf scour due to an *E. Coli* infection, according to research done by Vagh and Jani (2010) [22]. Research by Balikc**i** and Al (2014) on a sample of 40 diarrheal calves revealed that only 30% of the calves were infected with *E. Coli*. Many studies have shown that a variety of factors contribute to the differences in the relative percentages and prevalence of entero-pathogens that cause diarrhea in calves. These variables include the age of the animals (Krogh and Sherwood, 1983) [16], the parity of the dam from which the calf was borne the spatial distribution of diseases (Krogh and Sherwood, 1983; Garcia *et al*., 2000) [16, 11]. According to Fernandes *et al*. (2009) [8], the environment and water are major sources of *E. Coli* infection.

Table 1: bacterial isolates in the faecal samples of diarrhoeic calves

S. No.	Isolates	Samples (n=45)	Percentage
1	*Escherichia coli*	26	57.77
2	*Salmonella* spp.	08	17.78
3	*Escherichia coli* + *Salmonella* spp.	11	24.44

Table 2: Percentage of different bacterial isolates in the faecal samples of diarrhoeic calves

S. No.	Isolates	Total no. of isolates (n=45)	Percentage
1	*Escherichia coli*	32	71.11
2	*Salmonella* spp.	13	28.89

Summary and conclusion
Primary, secondary, and tertiary nutrient agar plates were streaked with the faecal samples of diarrheal calves obtained through high culture sterile swab to produce isolated colonies of bacteria. These petri plates were incubated for 24 hrs. at 37 ºC and isolated colonies were subcultured and incubated for 24 hrs. and 48 hrs. on Eosin Methylene blue agar (EMB) and XLD agar for isolation of *Escherichia coli* and *Salmonella* spp., respectively. The confirmation of the bacterial isolates was done by using biochemical test kits (HiEnterobacteriaceae Identification kit KB003). The research concludes that the most often isolated causal bacteria for calf diarrhea were *E. Coli* following *Salmonella* spp. A study revealed that the most common cause of diarrhea young calves was *E. Coli* spp.

Reference
1. Azizzadeh M, Shooroki HF, Kamalabadi AS, Stevenson MA. Factors affecting calf mortality in Iranian Holstein dairy herds. Prev Vet Med. 2012;104:335–340.
2. Aydin F, Umur S, Gocke G, Geno O, Guler MA. The isolation and identification of bacteria and parasites from diarrhoeic calves in Kars district. Kafkas Univ Vet Fak Derg. 2001;7(1):7-14.
3. Arora N, Rajora VS, Shukla SK, Singh JL. Evaluation of a poly-herbal formulation in the treatment of calf diarrhoea. Indian Vet J. 2010;87(10):1043-1044.
4. Ansari RAIH, Rahman MM, Islam MZ, Das BC, Habib A, Belal SM, *et al*. Prevalence and antimicrobial resistance profile of Escherichia coli and *Salmonella* isolated from diarrheic calves. J Anim Health Prod. 2014;2(1):12-15.
5. Asati CK, Roy S, Roy M. Hemato-biochemical study and diagnosis of colibacilosis in calves. Intas Polivet. 2008;9(2):245-248.
6. Chaurasia R. Characterization, enterotoxigenicity and transmissible drug resistance of *E. Coli* associated with calf diarrhoea. M.V.Sc. Thesis submitted to Rajasthan Agriculture University, Bikaner, India; c2001.
7. Cho Y, Yoon KJ. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. J Vet Sci. 2014;15(1):1–17.
8. Fernandes CE, Roy K, Malik YPS. Seroprevalence of Escherichia coli isolates from bovine neonatal enterocolibacillosis. Indian J Vet Med. 2009;29(1):39-40.
9. Gomez DE, Weese JS. Viral enteritis in calves. Can Vet J. 2017;58(12):1267-1274.
10. Ghanem MM, Fkhrany SF, Abd El-Roaf YM, El-Attar HM. Clinical and hematological evaluation of diarrheic neonatal buffalo calves (*Bubalus Bubalis*) with reference to antioxidant changes. Benha Vet Med J. 2012;23(2):275-288.
11. Garcia A, Ruiz-Santa-Quiteria JA, Orden JA, Sanz DC, Gomez-Bautista M, De la Fuenta FR. Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comp Immunol Microbiol Infect Dis. 2000;23:175-183.
12. Gay CC, Besser TE. Escherichia coli septicaemia in calves. In: Gyles CL, editor. Escherichia coli in Domestic Animals and Humans. 1st ed. CAB International; Wallingford, UK; 1994. p. 75.
13. Hansen J, Perry B. The Epidemiology, Diagnosis, and Control of gastrointestinal parasites of Ruminants of Africa. In Annual Report of International Laboratory for Research on Animal Disease, Narobi, Kenya. 1990. pp. 62-67.
14. Hansa A, Rai RB, Yaqoob Wani M, Dhama K. ELISA and RT-PCR based detection of bovine coronavirus in northern India. Asian J Anim Vet Adv. 2012;7:1120-1129.
15. Kumar BK, Shekher P, Kumar N. A clinical study on neonatal calf diarrhoea. Intas Polivet. 2010;11(2):233-235.
16. Krogh M, Sherwood D. Prevalence of enterotoxigenic *E. Coli* in calf diarrhoea in Northern England and Holland. Vet Rec. 1983;113:210-212.
17. Nayak TC, Singh AP, Maharishi T, Kachhawa JP, Gupta SR, Yadav R, *et al*. Diagnosis of colibacillosis in young calves by multiplex PCR. National Symposium on Holistic approach in Veterinary Medicine for better animal health to meet challenges of One health Mission. 2019. p. 311.
18. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Disease of the alimentary tract. A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th Edn. Saunders publication co., Oxford, London; c2007. pp. 92-93.
19. Ramkumar PK. Evaluation of therapeutic potential of plant extracts against *E. Coli* diarrhoea in calves. MVSc thesis, IVRI Deemed University, Izatnagar, India; c2012.
20. Sharma SK. Epidemiological, Clinical and Hemato-

biochemical Characterization of Calf Diarrhoea and Evaluation of Therapeutic Regimens. Ph.D. Thesis submitted to RAJUVAS, Bikaner (Rajasthan). 2013.

21. Singla LD, Gupta MP, Singh H, Singh ST, Kaur P, Juyal PD. Antigen-based diagnosis of *Cryptosporidium* parvum infection in cattle and buffalo faeces. Indian J Anim Sci. 2013;83(1):37–39.

22. Vagh AA, Jani RG. Prevalence and Comparative Studies of Some Major Serotypes of *E. Coli* from Cattle and Buffalo Calf Scour. Vet World. 2010;3(10):458-459.