

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; 8(1): 191-192
www.biochemjournal.com
 Received: 06-11-2023
 Accepted: 10-12-2023

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Bacterial studies in buffalo calves affected with diarrhoea (*Bubalus bubalis*)

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i1c.334>

Abstract

The primary goal of the study is to examine the bacteriological investigations linked with impacted diarrhoeic buffalo calves, regardless of age group, gender, or breed. Faecal swabs were collected aseptically from diarrheal calves for microbiological analysis. During the post-mortem A swab was obtained from affected parts of the gastro-intestinal tract that showed lesions under aseptic circumstances and delivered to the laboratory on ice. The swabs were then placed in a nutrient broth medium for further processing. This study was conducted on buffalo calves raised on private dairy farms as well as animals owned by individuals in the Bikaner region and surrounding areas of Rajasthan. This study includes sixty calves with diarrhea symptoms and twenty calves with no evidence of illness (controlled). The experimental research demonstrated that *E. coli*, *Klebsiella* spp., *Salmonella* spp., and *Staphylococcus* spp. are some of the most common bacteria discovered in diarrheic calves' feces.

Keywords: *E. coli*, *Klebsiella* spp, *Salmonella* spp., *Staphylococcus* spp., diarrhea

Introduction

Diarrhea is a major constraint on livestock productivity around the world. High rates of morbidity and death from gastroenteritis in newborn calves resulted in significant economic loss. The clinical state known as "calf scours" is associated with a variety of diarrhea-causing infections. Electrolyte imbalances can be harmful because the gut is unable to absorb fluids normally, resulting in dehydration, electrolyte loss, and acidosis. This illness causes rapid loss of body fluids because to scouring calfs. While infectious organisms may cause initial intestinal damage such as dehydration, acidosis, and electrolyte loss, which are more likely to be fatal. To develop effective preventative and treatment techniques, the pathogenic microorganisms that cause scours must be identified. Pathogenic *E. coli* serotypes are associated with colibacillosis, a major cause of diarrhea in newborn calves. *E. coli* is categorized into six pathogroups based on virulence: enterotoxigenic *E. coli* (ETEC), shiga toxin-producing *E. coli*, enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggressive *E. coli* (EAEC), and enterohaemorrhagic *E. coli* (EHEC). Nataro and Kaper (1998) [3].

Material and Methods

Sample collection for bacteriological studies

Faecal samples were obtained aseptically from diarrheal calves for microbiological analysis. During the post-mortem examination, a swab was collected from affected parts of the gastro-intestinal tract that showed lesions under aseptic circumstances and delivered to the laboratory on ice. The swabs were then placed in a nutrient broth medium for further processing.

Isolation and identification of bacteria

Culture examination of faecal samples was carried out for isolation of bacteria as per standard procedure of Cowan and Steel (1974) [1].

Primary Test for Identification of Bacteria

(a) Gram's staining (b) Catalase activity (c) Oxidase test

Colonial Characteristics of Bacteria on Specific/Selective agar Medium

Staphylococcal colonies were found on mannitol salt agar culture plates. Because of the high concentration of sodium chloride in the agar, the medium favors Gram-positive bacteria and inhibits the development of most other microorganisms. On mannitol salt agar, *Staphylococcus aureus* (Mannitol fermenting) developed mostly yellow colonies with some darker regions. The existence of colonies on plates of Mac-Conkey agar medium was also investigated. The lactose fermentation responses of these colonies were investigated further. Colonies that fermented lactose looked red or pink on the culture medium, whereas others were colorless. Now, pure lactose fermenting cultures were streaked over "Eosine Methylene Blue" (EMB) agar plates and incubated for 24 hours. The metallic sheen on EMB agar indicated the presence of *E. coli* colonies. Colonies with mucoid shape produced in various culture media were suspected to be *Klebsiella* spp. Faecal swabs were used to inoculate 10 ml of mannitol selenite broth, which was subsequently incubated at 37 °C for 24 hours. To isolate and identify pure *Salmonella* colonies, swabs were streaked onto "xylose lysine deoxycholate" (XLD) agar

plates and grown at 37 °C for 18-24 hours. *Salmonella* colonies on XLD agar exhibited a red outer ring and a black center.

Biochemical characterization (Secondary tests)

The bacteria responsible for calf diarrhoea were identified biochemically utilizing the "KB001 HiMVIC Biochemical test kit". Specific biochemical assessments for identifying each species of bacteria are included in each kit, along with a regulated colorimetric identification technique based on carbohydrate use.

Results and Discussion

Out of the 60 faecal samples, 24 exhibited bacterial growth on various media. According to culture and biochemical tests, the etiology of calf diarrhoea was caused by *E. coli* 19 isolates (79.16 percent), *Klebsiella* spp. 5 isolates (20 percent), *Staphylococcus* spp. one isolate (4.1%), and *Salmonella* spp. one isolate (4.1%). The highest prevalence of bacterial species was determined to be *E. coli*, followed by *Klebsiella* spp., *Staphylococcus* spp., and *Salmonella* spp.

Table 1: Culture characteristics of different bacteria

S. No.	Bacteria	Selective Media	Culture Characteristics
1.	<i>E. coli</i>	Eosin Methylene Blue	Metallic green sheen
2.	<i>Klebsiella</i> spp.	Mac-Conkey agar	Mucoid colonies
3.	<i>Staphylococcus</i> spp.	Mannitol salt agar	Yellow colonies with yellow zones
4.	<i>Salmonella</i> spp	Xylose Lysine Deoxycholate	Red colonies with black centres

Table 2: Primary Biochemical Test

S. No.	Bacteria	Gram's Staining	Catalase	Oxidase	Mortality
1.	<i>E. coli</i>	—	+	—	Motile
2.	<i>Klebsiella</i> spp	—	+	—	Non motile
3.	<i>Staphylococcus</i> spp	+	+	—	Motile
4.	<i>Salmonella</i>	—	+	—	Motile

Possible causes of the high prevalence of *E. coli* infections in cattle include insufficient colostrum feeding, inefficient antibody production, stress, and hormonal fluctuations that favor the development of sensitive bacteria. These findings were almost identical to those of Carlson *et al.* (2002) [2]; Singh (2003) [6]; Nidhi (2012) [4]; and Sarvan (2017) [5].

Conclusion

The current investigation demonstrated that *E. coli* was the most commonly isolated organism in diarrhoeic calves, followed by *Klebsiella* spp., *Salmonella* spp., and *Staphylococcus* spp.

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