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

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### Abstract

The primary objective of the study is to analyze the bacteriological studies associated with affected diarrhoeic buffalo calves irrespective age group, sex and breed. Faecal swab were collected aseptically from diarrhoeic calves for microbiological study. During post-mortem examination, a swab was taken from affected areas of gastro-intestinal tract showing lesions under aseptic conditions and transported on ice to laboratory. Then, swabs were placed in to nutrient broth medium for further process. This research work has been performed on Buffalo calves living in the private dairy farms and animals of personal holdings in the Bikaner region and neighboring parts of Rajasthan. This work comprises of Sixty calves with diarrhea symptoms and twenty calves with no signs of illness (Controlled). The experimental analysis revealed that some common bacteria found in the faecal samples of diarrheic calves are *E. coli*, *Klebsiella* spp., *Salmonella* spp., and *Staphylococcus* spp.

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

### Introduction

Worldwide, diarrhea is a significant barrier to livestock productivity. High rates of morbidity and death due to gastroenteritis in newborn calves resulted in substantial economic loss. The clinical state known as "calf scours" is linked to a number of illnesses that cause diarrhea. Electrolyte imbalances may be dangerous, when the gut is unable to absorb fluids normally which leads to dehydration, electrolyte loss, and acidosis. This condition results in fast loss of bodily fluid due to scouring calf's. While infectious organisms may be responsible for the first intestinal damage like dehydration, acidosis, and electrolyte loss which are more likely to be fatal. For effective prevention and treatment strategies, the infectious pathogens that cause scours must be identified. Pathogenic serotypes of *E. coli* are linked to colibacillosis, a common cause of diarrhea in newborn calves. There are six pathogroups of *E. coli* classified on the basis of virulence scheme such as "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 and Kaper *et al.*, 2004) [3, 7].

### Material and Methods

#### Sample collection for bacteriological studies

The samples of Faecal were collected aseptically from diarrhoeic calves for microbiological study. During post-mortem examination, a swab was taken from affected areas of gastro-intestinal tract showing lesions under aseptic conditions and transported on ice to laboratory. Then, swabs were placed in to nutrient broth medium for further process.

#### 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 (1975) [1].

#### Primary Test for Identification of Bacteria

- Gram's staining
- Catalase activity
- Oxidase test

#### Colonial Characteristics of Bacteria on Specific/Selective agar Medium

Staphylococcal colonies were seen on mannitol salt agar culture plates.

Because the medium is selective for Gram-positive bacteria due to the high quantity of sodium chloride contained in the agar, which inhibits the growth of most other bacteria. On mannitol salt agar, *Staphylococcus aureus* (Mannitol fermenting) formed colonies that were mostly yellow but had some darker areas. The presence of colonies on plates of Mac-Conkey agar medium was also analyzed. The lactose fermentation response of these colonies was analyzed further. Colonies that fermented lactose appeared red or pink on culture medium, otherwise it appeared colorless. Now, "Eosine Methylene Blue" (EMB) agar plates were streaked with pure lactose fermenting cultures and put in an incubator for 24 hours. Metallic sheen on EMB agar was indicative of *E. coli* colonies. Colonies with mucoid morphology grown in other culture media were suspected of being *Klebsiella* spp. Swab samples of faecal were used to inoculate 10 ml of mannitol selenite broth, which was then incubated at 37 °C for 24 hours. For the purpose of isolating and identifying pure colonies of *Salmonella*, the swabs were streaked onto "xylose lysine deoxycholate" (XLD) agar plates and cultured at 37 °C for 18-24 hours. *Salmonella* colonies on XLD agar had 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 these 60 faecal samples, 24 showed bacterial growth on different media. The etiological prevalence of calf diarrhoea caused by different bacteria were *E.coli* 19 isolates (79.16 percent), *Klebsiella* spp. 5 isolates (20 per cent), *Staphylococcus* spp. one isolates (4.1 per cent) and *Salmonella* spp. one isolates (4.1 per cent) according to culture and biochemical test either as single or mixed pathogens. Highest prevalence of bacterial species was found to be of *E. coli* followed by *Klebsiella* spp., *Staphylococcus* spp. and *Salmonella* spp. respectively.

**Table 1:** Culture characteristics of different bacteria

S. No.	Bacteria	Selective Media	Culture Characteristics
1.	<i>E. coli</i>	Eosine 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 for the high prevalence of *E. coli* infections was studied for bovines that include inadequate colostrum feeding, ineffective antibody generation, stress, and hormonal variations that favor the development of vulnerable bacteria. These results were almost consistent with the findings of Carlson *et al.* (2002) [2]; Singh (2003) [6], Nidhi, (2012) [4] and Sarvan (2017) [5].

### Conclusion

From the present study we revealed that maximum isolates in diarrhoeic calves was of *E. coli* followed by *Klebsiella* spp., *Salmonella* spp., and *Staphylococcus* spp.

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