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Biochemical characterization of *Salmonella species* isolated from calf diarrhoea

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Abstract
The dairy sector and farms have been seriously threatened by calf diarrhea. One of the main causes of this multi-etiology sickness is *Salmonella* spp. The goal of this work is to characterize *Salmonella* spp. that have been isolated from calf diarrhea biochemically. A total of 100 fecal samples were collected from calves of cattle and buffalo of below 3 months of age. Sterile fecal swabs (Hi-media, India) were used for sample collection. All the samples were transported immediately to the laboratory under cold chain and were processed for biochemical characterization of *Salmonella* spp. For isolation and identification of *Salmonella* spp. a loopful of fecal sample was enriched in Selenite broth and incubated at 42 °C for 12-14 hours. After that a loopful of selenite broth was inoculated and streaked separately onto selective agar plates as xylose Lysine Deoxycholate (XLD) agar, Brilliant Green agar (BGA), MacConkey's agar and incubated at 37 °C for 24hours. Partially identified cultures were suspended in normal saline and smeared over a slide. The smears were allowed to air dry followed by heat fixing and Gram's staining. All Gram negative culture was subjected to biochemical characterization by using various biochemical tests such as catalase, oxidation, oxidation-fermentation, Indole, methyl –red, Voges-Poskauer, citrate test, TSI and urease test. The overall prevalence of *Salmonella* spp. in the present study was recorded 3%.

Keywords: *Salmonella species*, isolated, calf diarrhoea

Introduction
Salmonellosis is a zoonotic disease that can infect both calves and adults, resulting in serious sickness. Bovine Salmonellosis symptoms include diarrhoea, fever, anorexia, dehydration, abortion and endotoxemia (Divers and Peek, 2008) [1]. Owing to mortality, treatment costs reduced the herd's weight increase and milk production, and the risk of human infection through the food chain or direct animal contact, bovine for dairy farmers, salmonellosis is an expensive illness (Lorenz *et al.*, 2011) [8]. Humans become sick by eating undercooked or contaminated food and *Salmonella* is commonly found in cattle (Majowicz *et al.*, 2010) [9]. Animal feces carry the enteric infection *Salmonella*, which lives in the intestines of animals and infects people. It can be spread by water, plant surfaces, soil, animal excrement, and dairy farms (Halimi *et al.*, 2014) [10].

Material and methods
In the present study, attempts were made to determine the isolation and characterization of *Salmonella* spp. associated with calf diarrhea.

Isolation and identification of *Salmonella* spp. from collected samples
Isolation of *Salmonella* strains from the 100 samples collected in and around College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur were conducted. The samples (25 ml/gm) were homogenized with 225 ml of buffered peptone water (BPW) in a sterile culture flask to obtain 1 part sample + 9 parts buffered peptone water and was incubated at 37 °C for 24 hours for pre-enrichment. Following incubation, 1 ml of inoculum was transferred into 10 ml of Selenite broth medium for enrichment and further incubated at 42 °C for 12-14 hrs.

Colony characteristics and morphology of *Salmonella* spp.
A loopful of the inoculum from selenite broth was streaked on MCA agar, brilliant green agar (BGA) and xylose lysine deoxycholate agar (XLD). Further, identification of *Salmonella* spp. was done on the basis of cultural, morphological and biochemical methods. These putative isolates were further confirmed on the basis of their morphology by Gram's staining.

Biochemical characterization
The primary biochemical identification of *Salmonella* spp. was done by Gram's staining, catalase test, oxidase test and oxidation fermentation (OF) test then the bacterial strain were confirmed by secondary biochemical test *viz.*, Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, triple sugar iron agar test, Urea hydrolysis test and H_2S production (Quinn *et al.*, 2004 and Andrews *et al.*, 1998) [11, 12].

Result and discussion
In the present investigation 100 calves diarrhoeic samples were collected and cultured for primary isolation of *Salmonella* spp. Three samples suspected for *Salmonella* spp. were further characterized on the basis of morphology, cultural and biochemical characterization. The overall prevalence of *Salmonella* spp. in the present study was recorded 3%.

Isolation and cultural characterization of *Salmonella* spp.
For isolation of *Salmonella* spp. a loopful of the faecal sample was enriched in Selenite broth and incubated at 42 °C for 12-14 hours. After that a loopful of selenite broth was inoculated and streaked separately onto MacConkey's agar and after that on selective agar plates as xylose Lysine Deoxycholate (XLD) agar and Brilliant Green agar (BGA) incubated at 37 °C for 24 hours.
On MacConkey agar plates, non-lactose fermenting, colourless and transparent colonies were observed (fig. 4). On brilliant green agar plates changed in the colour of the agar plates and pink to Red coloured colonies were seen (fig. 5). On Xylose Lysine Deoxycholate agar red colonies with black center observed (fig. 6).
On gram's staining, the morphology of the isolates was gram-negative, pink colour small rod shape (fig. 2). On the basis of these cultural characteristics, 3 samples were considered as *Salmonella* spp. and further confirmed by biochemical characteristics.

Biochemical characterization
All the isolated *Salmonella* strains were found catalase positive (fig. 1a), oxidase negative (fig.1b) and fermentative in OF test (fig. 3). The results of IMViC test were (-/+/-/+) with Indole test negative, Methyl Red test positive, Voges Proskauer test negative and Citrate utilization test positive (fig. 8). On TSI agar slant all three strains produced red slants (alkaline) and yellow butts (acid) along with the blackening of the agar due to formation of hydrogen sulphide (fig.7). On urea agar slant all strains gave negative results as no change of colour was observed (fig. 9).

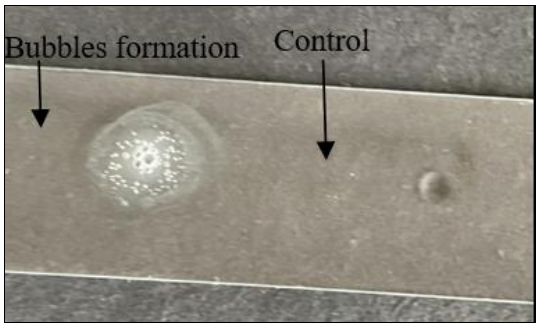

Fig 1a: Primary characterization of *Salmonella* spp. (a) catalase +ve

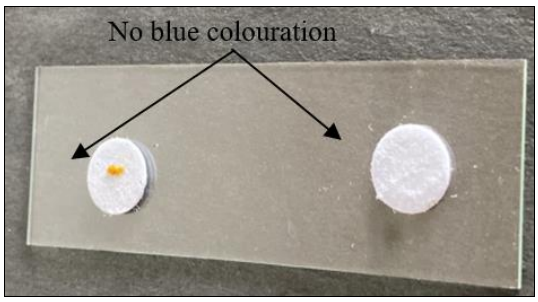

Fig 1b: oxidase-ve

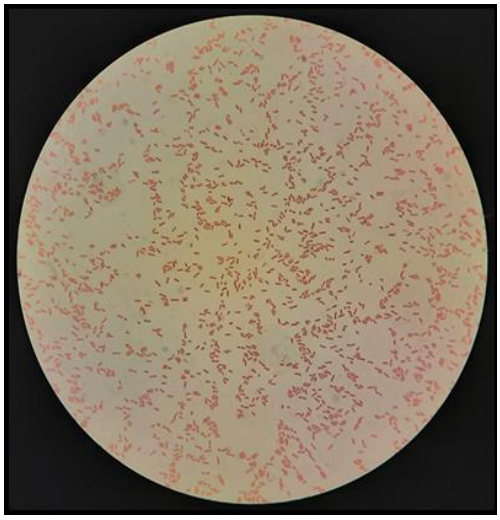

Fig 2: Gram's staining

Fig 3: Oxidation–fermentation test

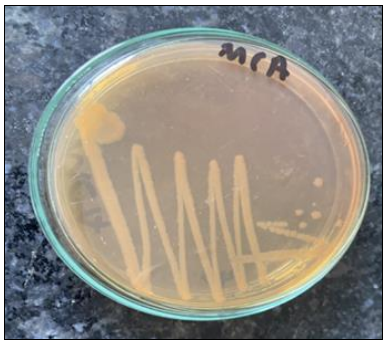

Fig 4: Cultural characteristics of *Salmonella* spp. on MacConkey Agar

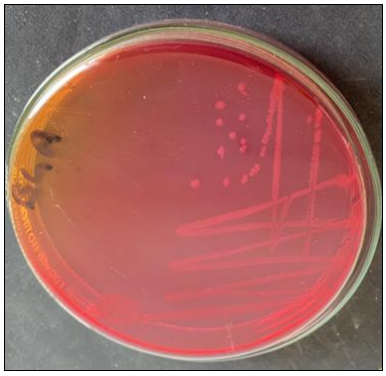

Fig 5: Brilliant Green Agar

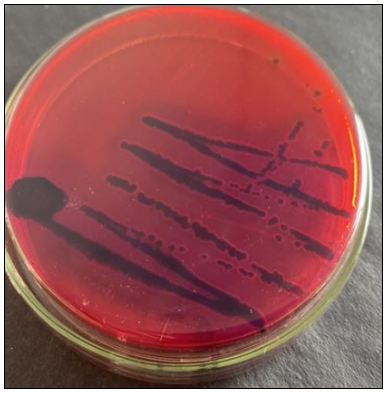

Fig 6: Xylose Lysine Deoxycholate Agar

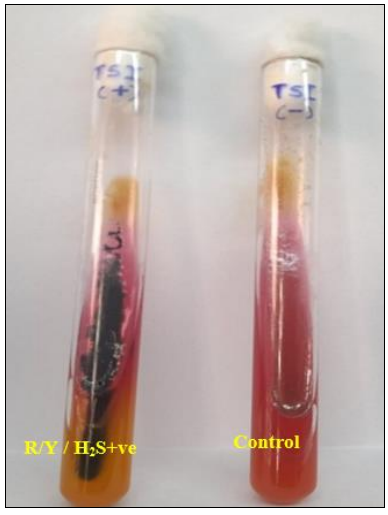

Fig 7: Triple Sugar Iron test

Fig 8: IMViC test

Fig 9: Urease test

In present study is in close concordance with the earlier workers who studied and reported the prevalence of *Salmonella* 2% by (Achaa *et al.*, 2004) [21]; 4.09% by (Younis *et al.*, 2009) [22]; 4.02% by (Rana *et al.*, 2012) [19]; 1.87% by (El Sherbiny *et al.*, 2016) [18]; 3.3% by (Awosile *et al.*, 2018) [15] and 2.71% by (de Vasconcelos *et al.*,2021). While higher incidences of *Salmonella* isolated from diarrhoeic calves were also described by (Anwarullah *et al.*, 2014; Zahran and El-Behiry, 2014; Olaogun *et al.*, 2016 and Sohidullah *et al.*, 2016) [14, 20, 13, 16] with isolation rates of 18.6%,9.75% 52.6% and 14.03%, respectively. The lower prevalence of *Salmonella* spp. might be indicating the better management for the investigated calf, small herd size and the geographical area of the study. *Salmonella* spp. was the most common

isolates from diarrhoeic calves samples and many previous reports supported our study (Abdullah *et al.*, 2013; El-seedy *et al.*, 2016 and Moustafa *et al*., 2020) [3, 4, 5]. Therefore, the hygiene of the cattle farms should be improved to prevent and control calf enteritis. The difference in prevalence rates of diarrhoeal calves due to Salmonellosis compared to previous studies can be explained by factors such as insufficient hygiene measures, particularly with regard to faecal management, due to stress, the non-segregation of animals by age and keeping sick animals in contact with healthy animals (Al Mawly *et al.*, 2015) [6]. Similar reports on specific identification and biochemical characterization of *Salmonella* from diarrhoeic calves were also observed by El-Twab *et al.*, 2016a and Aziz *et al.*, 2018 [7].

Conclusion

Farmers depend on animal husbandry to sustain their social status. Out of all the animal husbandry jobs, dairy farming is the most lucrative. Dairy farmers are significantly impacted by several diseases that afflict the cattle industry. The multifactorial condition known as calves enteritis is caused by bacteria, viruses, parasites, and other infectious and non-infectious causes. These microorganisms can all cause enteritis, either separately or in combination. These pathogens include bacteria such as *Salmonella* spp., *E. coli, Clostridium* spp., protozoa such as *Eimeria* spp. and viruses. *Salmonella* is found throughout countryside and causes a variety of infections in humans and animals.

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