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Short Communication

MOLECULAR SCREENING OF *SALMONELLA* SPP. IN BUFFALO CALVES FROM AN ORGANISED DAIRY FARM

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SUMMARY

An examination of 38 faecal samples using Polymerase chain reaction (PCR) from buffalo calves with and without diarrhoea for detection of Salmonella genes, revealed the detection of a total of 13 genus specific (16SrRNA) genes which accounts for 11 (28.95%) positive samples. Despite low Salmonella detection rate, the number may still contribute to occurrence of diarrhoea in these animals. It is therefore suggested that larger samples should be collected for a wider screening.

Keywords: Buffalo Calves, Diarrhoegenic, Non-diarrhoegenic, Faeces, PCR, Salmonella

In bovines, *Salmonella* infections comprise the second most economically important bacterial disease affecting the gastrointestinal system following *E. coli* infections. (Ekperigin and Nagaraja, 1998). Despite the fact that most bacterial pathogens can be easily cultured, there are a number of problems associated with attempts to detect and quantify them. Consequently, rapid, accurate and culture-independent alternatives are being investigated to enable detection of pathogens in diarrhoea cases (Alexandrino *et al*., 2004). Different molecular typing methods based on variations in genetic makeup have been now used in complement with traditional typing methods for fingerprinting of *Salmonella* serotypes. The present study was aimed at molecular screening of faecal samples from buffalo calves for *Salmonella* spp. (Rakesh *et al*., 2009).

Faecal samples from 38 buffalo calves were collected from buffalo farm in Lala Lajpat Rai, University of Veterinary and Animal Sciences (LUVAS), Hisar. Buffalo calves were grouped on the basis of age *viz*. Group1 (day1 to day2) Group2 (Day 3 to 3 months) and Group3 (4 to 6 months). Twenty of 38 calves were diarrhoeic and 18 were healthy clinically.

One gram of each faecal sample was processed for extraction of DNA to detect various genes in Polymerase chain reaction (PCR) assays (Invitrogen, 2018).

PCR was carried out using primer pair Sal-F (5' - TGTTGTGGTTAATAAC CGCA-3') and Sal-R (5'- CACAATCCATCTCTGGA-3') for amplifying (16s rRNA) coding gene complicon size 544 bp supplied by Thermo® (USA). Thermal cycle comprised of initial denatuvation at 98 ºC for 3 min followed by 40 cycle of denaturation (98 ºC for 1 min), annealing (51.4 ºC for 45 sec.); extension (72 ºC for 2 min) and final extension at 72 ºC for 10 min.

The PCR assays were carried out in 25 µl reaction volume containing 1U of Taq polymerase, 200 µmol of each dNTP and 2.5 µl of 10× PCR buffer. Reactions were performed in a GeneAmp® PCR System 2400 Thermal Cycler (Applied Biosystems, California) and FlexiGene® thermal cycler (Techne Inc., New Jersey).

An examination of 38 faecal samples from buffalo calves revealed that 11 were positive for salmonella using genus specific genes. Chi-square analysis of Salmonella positivity was assessed for effect of presence of diarrhoea, age and sex and was found non-significant (P>0.05).

The present study was conducted to detect the presence of Salmonella infection from diarrhoeic and non-diarrhoeic buffalo calves using polymerase chain reaction and recorded the prevalence of 28.95 (11/38).

Similar observations were recorded by Fahmy *et al.* (2017) after examining faeces of calves from feedlot farms in Egypt but the percentage of *Salmonella* isolated from diarrhoeic calves was 7%. The findings of El-Shehedi *et al.* (2015) who reported the prevalence of *Salmonella* infection after examining faecal samples from diarrhoeic calves in Egypt was also lower compared with the findings in the present study. Various workers reported varying degree of prevalence of *Salmonella* from diarrhoeic faeces *viz.* 4.0% (Haggag and Khaleel, 2002); 4.09% (Younis *et al.,* 2009); 1.8%, (Garcia *et al.*, 2000); 2.0% (Acha *et al.,* 2004) and 1.56% (Osama *et al.,* 2011). The variations in prevalence of *Salmonella* among apparently healthy and diarrheic calves reported in different countries reflect the effect of wide range of different management risk factors (Vanselow *et al*., 2007 & Jones, 2011). Wani *et al*., 2013, however reported

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Escherichia coli but no *Salmonella* from the faecal samples collected from buffalos' calves of Kashmir region in India. This was further supported by reports from other parts of India and Mozambique according to Hussain and Saikia (2000) and Acha *et al.* (2004), respectively who in their separate studies recorded negative outcomes. Despite low *Salmonella* detection rate, the number may still contribute to occurrence of diarrhoea in these animals. It is therefore suggested that larger samples should be collected for a wider screening.

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