The Pharma Innovation Journal 2020; 9(9): 287-290

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(9): 287-290

www.thepharmajournal.com
Received: 27-06-2020
Accepted: 16-08-2020

M Srivani
Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

Y Narasimha Reddy
Department of Veterinary Microbiology, College of Veterinary Science, Rajendranagar, Rangareddy District, Telangana, India

KV Subramanyam
Department of Veterinary Microbiology, College of Veterinary Science, Proddatur, Andhra Pradesh, India

M Lakshman
Roska lab, College of Veterinary Science, Rajendranagar, Rangareddy District, Telangana, India

KL Kavitha
Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

RN Ramani Pushpa
Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

Corresponding Author:
M Srivani
Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, Krishna District, Andhra Pradesh, India

Epidemiology, molecular characterization and antimicrobial resistance of Necrotoxigenic *Escherichia coli* in diarrhoeic calves

M Srivani, Y Narasimha Reddy, KV Subramanyam, M Lakshman, KL Kavitha and RN Ramani Pushpa

DOI: https://doi.org/10.22271/tpi.2020.v9.i9d.5125

Abstract
A study was carried out to investigate the epidemiology, molecular characterization and antibiotic resistance of Necrotoxigenic *E. coli* (NTEC) isolated from diarrhoeic calves in Andhra Pradesh (AP) and Telangana States (TS). A total of 129 faecal samples from diarrhoeic buffalo calves of 1 to 90 days were collected from various districts in AP and TS, of which 60 *E.coli* were isolated. The *cnf2* gene was detected by PCR and *In vitro* antibiotic susceptibility was tested by disk diffusion methods. The prevalence of *E.coli* associated diarrhea in calves was 46.51% of which 5.0% was due to NTEC based on the presence of *cnf*2 gene and none of the *E.coli* isolates possessed *cnf*1 gene. The NTEC isolates from diarrhoeic calves showed higher antibiotic resistance to tetracyclin and aztreonam (66.67%) and sensitive to ampicillin, cefotaxime, ceftazidime, amoxycillinclavulinicacid, gentamycin, kanamycin, streptomycin, sulfisoxazole, cotrimoxazole, ciprofloxacin, chloramphenicol and imipenem antibiotics. The present study provides baseline data on epidemiology of *E.coli* associated diarrhoeia, NTEC prevalence and antimicrobial resistance in calves which will help in formulating prophylactic and preventive measures in this geographic region.

Keywords: Epidemiology, molecular characterization, antimicrobial resistance, NTEC, calves

1. Introduction
*Escherichia coli*is a primary pathogen of calves causing neonatal diarrhoea and economic loss to the dairy producers. Epidemiological studies in calves have revealed that *E. coli* was the major cause of neonatal diarrhoea (Fagiolo *et al*., 2005 and Foster and Smith, 2009) [10, 11]. The mortality rate is high, particularly in calves of less than 3 months age in India (Tiwari *et al.*, 2007) [20]. The strains of *E. coli* that can able to produce a toxin called cytotoxic necrotising factor (CNF) are defined as Necrotoxigenic *E. coli* (NTEC). There were two kinds of cytotoxic necrotising factors (CNF1 and CNF2), causing urinary tract infection (UTI), septicaemia and diarrhoea in humans and animals (Orden *et al.*, 2002) [16]. However, to the best of our knowledge, no studies have been conducted so far on the prevalence of NTEC in calves of this zoographic area. Therefore, the present research has been undertaken to investigate the prevalence, molecular characterization and antibiotic resistance pattern of NTEC in diarrhoeic calves of Andhra Pradesh and Telangana States.

2. Materials and Methods
2.1 Sample collection
A total of 129 faecal samples from diarrhoeic calves of 1 to 7, 8-30, 31-60 and 61-90 day age groups were collected randomly from organized dairy farms and individual farmers of East Godavari, West Godavari, Krishna, Chittor, Districts of Andhra Pradesh State and Ranga Reddy and District of Telangana State. Geographical distribution and age of diarrhoeic calves were recorded during sampling. Fecal samples were collected using sterile rectal swabs. After collection, the swabs were immediately transported to the department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram in ice-cooled containers for *E. coli* isolation. All the samples were inoculated on to Maconkeys agar and incubated at 37°c for 24 hours. The pink colonies obtained were again inoculated in EMB agar and the colonies showing green metallic sheen were selected and confirmed as *E.coli* by standard biochemical

tests (Cruickshank 1970)[9]. Bacterial DNA was obtained by boiling the cells at 100^0 C for 15 min and then pelleting the cells by centrifugation. The supernatant was then used in the PCR reaction.

2.2 Detection of *cnf2* gene:

Table 1: Details of the primers used for the detection of *cnf*2gene

Primer	Sequence (5'---- 3')	Target gene	Expected Amplicon size(bp)	Reference
Cnf2-F	A AATCTAATTAAAGAGAAC	*cnf*2	543	Blanco *et al.* (1996) [6]
Cnf2-R	CATGCTTTGTATATCTA			

2.3 Standardization of PCR protocols for detection of *cnf*2gene

PCR for amplification of genes was set up in 25µL reaction (Eppendorf thermal cycler). Following initial trials with varying concentration of components the reaction mixture was optimized as below

Table 2: Composition of PCR reactions for virulence genes

Gene	Mastermix µL	Forward primer (20picomoles/ µL) µL	Reverse primer (20picomoles/µl) µL	Template µL	MgCl2 µL	NFW µL
cnf2	12.5	0.62	0.62	3.00	-	8.26

Table 3: PCR conditions for detection of virulence genes

Gene	Initial denaturation (°C/min)	Denaturation (°C/sec)	Annealing (°C/sec)	Extension ((°C/sec)	Final extension (°C/min)	No of cycles
cnf2	94/2	94/60	48/60	72/60	72/7	30

2.4 Antibiotic resistance

Antibiotic resistance against 18 different antibiotics was studied by disk diffusion method (Bauer *et al*., 1966) [4]. The diameter of the zone of inhibition was compared with the standard known value against each specific antimicrobial agent as suggested in the product information (interpretation guideline) from manufacturer.

3. Results and discussion

Out of the 129 faecal samples collected from diarrhoeic calves, 60 (46.51%) samples were found positive after biochemical characterization for *E. coli* (Table1). Among different age groups, highest (51.52%) prevalence of *E.coli* associated diarrhea was observed in 1-7 day age calves followed by 46.67% and 44.0% recoded in 8-30 and 31-60 day age groups, while lowest (36.36%) prevalence was observed in 61-90 day age calves.

Table 4: Fecal samples collected from diarrhoeic calves of different districts in Andhra Pradesh and Telangana states

District	Age of the calves				
	1-7	8-30	31-60	61-90	Total
East Godavari	5	6	4	2	17
West Godavari	5	8	5	2	20
Krishna	6	13	6	3	28
Chittor	11	23	6	2	42
Ranga Reddy	6	10	4	2	22
	33	60	25	11	129

Higher prevalence of *E. coli* associated diarrhoeia in the first week of calf age reported in the present study was also underlined by several investigators (Islam *et al*., 2015) [13] and Shahrani *et al*. 2014) [19] in various studies and calves of 1 day to 8 weeks old are highly susceptible to *E. coli* infection compared to older calves (Paul *et al*. 2010) [17].The primary determinant of the *E. coli* infection is deficiency of circulating immunoglobulins as the result of failure in passive transfer of colostral immunoglobulin particularly in calves that deprived of colostrum immediately after birth. The septicemia of *E. coli* is seen during the first weeks of life, with the highest incidence in calves of 2 to 5 days old (Bashahun and Amina, 2017) [3].

Table 5: *E.coli* isolated from the fecal samples of diarrhoeic calves obtained from different districts of Andhra Pradesh and Telangana states

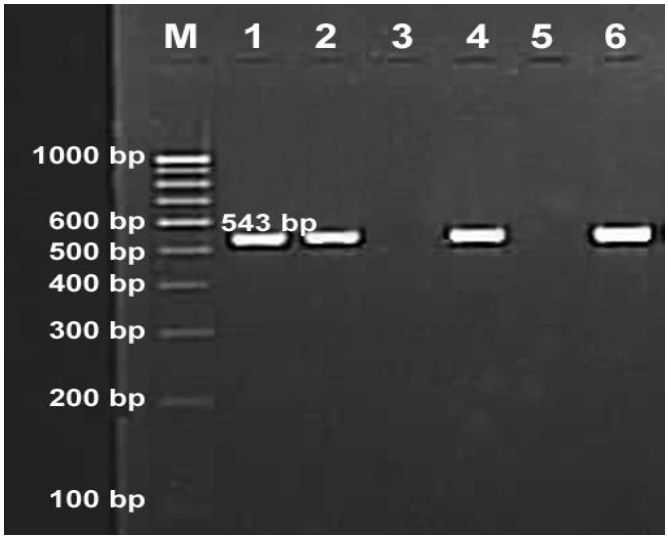
District	Age of the calves									
	1-7	%	8-30	%	31-60	%	61-90	%	Total	%
East Godavari	4	80	2	33.33	1	25.00	1	50.00	8	47.10
West Godavari	3	60	5	62.50	3	60.00	1	50.00	12	60.00
Krishna	3	50	6	46.15	3	50.00	-	-	12	42.85
Chittor	5	45.45	11	47.83	3	50.00	1	50.00	20	47.62
Ranga Reddy	2	33.33	4	40.00	1	25.00	1	50.00	8	36.36
	17	51.52	28	46.67	11	44.00	4	36.36	60	46.51

The higher prevalence of *E. coli* in younger calves may be due to poor managemental practices and predisposing factors like overcrowding and malnutrition, which are supposed to be a primary cause of immunosuppression (Abdulgayeid *et al.*,

2015) [1]. Further, *E. coli* is a commensal organism and is responsible for diarrhoea in calves, particularly calves receiving less or no maternal antibodies through colostrum where milk is mainly used for commercial purposes (Malik *et al*., 2013) [15].

Among the districts, high prevalence of *E. coli* associated diarrhoeia in calves was observed in West Godavari district (60%) of Andhra Pradesh, while it was low in Ranga Reddy district (36.36%) of Telangana state. The variation in the prevalence of *E. coli* associated diarrhoeia may be due to differences in preventive health management practices received by the calves.

The present study detected 5% of NTEC among the *E.coli* isolates from diarrhoeic calves based on the presence of *cnf*2 gene (Fig. 1).



Lane M: 100 bp DNA ladder
Lane 1, 2, 4, 6:*E.coli* isolates carrying *cnf*2 gene
Lanes 3, 5, Negative isolates

Fig 1: Amplified product of *cnf*2 gene

The predominance of *cnf*2 gene in NTEC isolated from calves as reported in this study is corroborated by earlier studies of Mahanti *et al.* (2014) [14] and Borriello *et al.*(2012) [7] in calves of India and Italy, respectively. The prevalence of NTEC found in this study (5%) was lower compared to the earlier reports by Borriello *et al.* (2012) [7] (20.9%) and Coura *et al.* (2019) [8] (19%) in diarrhoeic calves of Italy and Barzil, respectively and Rehman and Deka (2012) [18] (35.3%) in diarrhoeic calves of North East region of India. Contrary to the present study, lower prevalence of NTEC (1.9%) was reported by Mahanti *et al.* (2014) [14] in buffaloes of West Bengal, India. Blanco *et al*. (1996) [6] suggested that NTEC may form part of the normal intestinal flora in cattle. These differences in prevalence of NTEC may be due to differences in geographical regions and management practices received by the animals. The NTEC carrying *cnf*1 gene also has been found to be responsible for human diarrhoea worldwide (Bekal *et al*., 2003) [5]. However, in India NTEC carrying both *cnf*1 and *cnf*2 genes are found to be associated with childhood diarrhoea (Kavitha *et al*., 2010) [12]. Therefore, the present study revealed prevalence of NTEC in diarrhoeic calves which may pose zoonootic threat in this geographic region.

The NTEC isolates in the present study were found to have different degrees of resistance towards various antimicrobial agents. The NTEC isolates from diarrhoeic cow calves showed higher antibiotic resistance to tetracyclin and aztreonam (66.67%) and 100% sensitive to ampicillin, cefotaxime, ceftazidime, amoxycillinclavulinicacid, gentamycin, kanamycin, streptomycin, sulfisoxazole, cotrimoxazole, ciprofloxacin, chloramphenicol and imipenem antibiotics.

The increased antimicrobial resistance showed by NTEC for tetracycline was also reported earlier by Rehman and Deka (2012) [18] and Mahanti *et al.* (2014) [14] in diarrhoeic calves and buffaloes, respectively. The variations observed in the sensitivity to different antimicrobial agents may be due to the differences in the strains present in different regionsand may become resistant to antimicrobial agents used in that particular region.

The present study provides data on epidemiology of *E.coli* associated diarrhoeia and NTEC prevalence in calves which will help in formulating prophylactic and preventive measures in the geographic area studied. Further, diarrhoeic calves may be act as reservoirs of NTEC in this geographic area.

4. References

1. Abdulgayeid M, Hazem Shahin, Seham Foad, Madiha S, Ibrahim. Molecular characterization of *Escherichia coli* isolated from buffalo calves in El-Behera Governorate. Alexa. J of Vety. Sci. 2015; 47(1):90-96.
2. Abubaker A, El-Ayis, Ali A, Elgaddal, Yassir A, Almofti. Isolation, identification andenterotoxin detection of *Escherichia Coli* isolated from calf diarrhea and their virulence Characteristics. J of Appl. and Indus. Sci. 2015; 3(4):141-149.
3. Bashahun GM, Amina A. *Colibacillosis* in calves: A review of literature. J. of Anim. sci. and Vet. Medi. 2007; 2(3):62-71.
4. Bauer AW, Kirby MM, Sherris JC, Truck M. Antibiotic susceptibility testingby a standardized single disk method. American J of Clin. Pathol. 1996; 45(4):493-496.
5. Bekal SR, Brousseau L, Masson G, Prefontaine J, Fairbrother J, Herel. Rapid identifi cation of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. J. Clin. Microbiol. 2003; 41:2113-2125.
6. Blanco M, Blanco JE, Blanco J, Alonso MP, Balsalobre C, Mouriiio M *et al*. Polymerase chain reaction for detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 and 2 (CNFl and CNF2). J of Microbiol. Meth. 1996; 26:95-101.
7. Borriello G, Lucibelli MG, De Carlo E, Auriemma C, Cozza D, Ascione G *et al*. Characterization of enterotoxigenic *E. coli* (ETEC), Shiga-toxin producing *E. coli* (STEC) and necrotoxigenic *E. coli* (NTEC) isolated from diarrhoeic Mediterranean water buffalo calves (*Bubalusbubalis*). Research in Vet. Sci. 2002; 93(1):18-22.
8. Coura Fernanda Morcatti, Soraia de AraújoDiniz, Marcos Xavier Silva, Cairo Henrique Sousa de Oliveira, Jamili Maria Suhet Mussi, Camila Stefanie Fonseca de Oliveira *et al*. Virulence factors and phylotyping of Escherichia coli isolated from non-diarrheic and diarrheic water buffalo calves. Ciência Rural. 2019; 49:(5):2019.
9. Cruickshank R. Medical Microbiology. 11th ed. The English Language Book Society E and Livingston Ltd., Edinburgh, 1970.
10. Fagiolo A, Roncoroni C, Lai O, Borghese A. Buffalo Pathologies. In:Borghese, A.(Ed.), Buffalo Production and Research. FAO Regional Office for Europe Inter

Regional Cooperative Research Network on Buffalo, Rome, 2005, 249-296.
11. Foster DM, Smith GW. Pathophysiology of diarrhea in calves. Vet. Clinics of North America: Food Animal Practice. 2009; 25:13-36.
12. Kavitha K, Prabhakar K, Rajendran S, Uma B, Sarayu YL. Isolation of necrotoxigenic*Escherichia coli* from paediatric patients with acute diarrhea. J of Medical Microbiol. 2010; 59:503-504.
13. Islam KMA, Rahman M, Nahar A, Khair A, Alam MM. Investigation of pathogenic *Escherichia coli* from diarrheic calves in selective area of Bangladesh. Bangladesh J of Vet. Medi. 2015; 13(1):45-51.
14. Mahanti A, Samanta I, Bandyopadhyay S, Siddhartha NJ, Tapan K, Dutta TK *et al*. Isolation, molecular characterization and antibiotic resistance of Enterotoxigenicnterotoxigenic *E. coli* (ETEC) and Necrotoxigenic (ETEC) from healthy water buffalo. VeterinarskiArhiv. 2014; 84(3):241-250.
15. Malik S, Amit Kumar, Amit Kumar Verma, Manoj Kumar Gupta, SomDutt Sharma, Arvind Kumar Sharma *et al*. Incidence and drug resistance pattern of collibacillosis in cattle and buffalo calves in western Utter Pradesh in India. J of Anim. Health and Prod. 2013; 1(2):15-19.
16. Orden JA, Cid D, Ruiz-Santa-Quiteria JA, Garcia S, Martinez S, Fuente R de la. Verotoxin-producing *Escherichia coli* (VTEC), enteropathogenic *Escherichia coli* (EPEC) and necrotoxigenic *Escherichia coli* (NTEC) isolated from healthy cattle in Spain. J of Applied Microbiology. 2002; 93(1):29-35.
17. Paul SK, Khan MSR, Rashid MA. Hassan J, Mahmud SMS. Isolation and characterization of *Escherichia coli* from buffalo calves in some selected areas of Bangladesh Bangladesh. J. of Vete. Medi. 2010; 8(1):23-26.
18. Rahman H, Deka M. Detection and Characterization of Necrotoxin Producing Escherichia coli (NTEC) from Calves with Diarrhoea. Indian J Med Res. 2014; 139(4):632-637.
19. Shahrani M, Farhad Safarpoor Dehkordi, Hassan Momtaz. Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol. Res. 2014; 47(1):1-13.
20. Tiwari R, Sharma MC, Singh BP. Buffalo calf health care in commercial dairy farms: A field study in Uttar Pradesh (India). Livest. Res. Rural Dev. 2007; 19(3):38.