www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 781-784

www.thepharmajournal.com
Received: 01-08-2023
Accepted: 05-09-2023

Lahari L
State Level Disease Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Sravanthi M
State Level Disease Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Rani Prameela D
State Level Disease Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Corresponding Author:
Lahari L
State Level Disease Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Isolation and toxinotyping of *Clostridium perfringens* from piglets

Lahari L, Sravanthi M and Rani Prameela D

Abstract
Clostridium perfringens is a pathogen causing various digestive system diseases such as diarrhoea, hemorrhagic and necrotic enteritis in piglets. The present study aimed at isolation and toxinotyping of *Clostridium perfringens* from piglets. Two *Clostridium perfringens* isolates were obtained from bloody diarrheic piglets ageing 3 months old. The isolates were found to be *Clostridium perfringens* type C using multiplex PCR assay. On PCR, α toxin gene (cpa) and β toxin (cpb) gene were amplified confirming the isolates as *Clostridium perfringens* type C.

Keywords: *Clostridium perfringens*, multiplex PCR, piglets, toxin genes

Introduction
Clostridium perfringens is present in the soil and in the intestinal contents of healthy animals and humans [1]. *Clostridium perfringens* type C is considered to be the major cause of necrotic enteritis in newborn piglets of 0-2 week [2]. The pathogenecity of the organism is due to various toxins based on which toxinotyping of the bacteria is done. *Clostridium perfringens* type C producing α- toxin and β- toxin can be found in the intestinal tract of healthy animals, but due to the predisposing risk factors organisms proliferate and induce disease under appropriate conditions. Some strains produce enterotoxin encoded by cpe gene [3]. *Clostridium perfringens* type C infection occurs world wide causing Haemorrhagic, often fatal necrotic enteritis in young piglets [4].
Clostridium perfringens type C disease in pigs is an economically important disease because the mortality rates are upto 100% in affected piglets [5]. Acute cases of *Clostridium perfringens* with hemorrhagic diarrhoea occurs shortly before the death. The β-toxin of *Clostridium perfringens* type C quickly destroys the epithelial lining of the small intestine and blood is lost into the intestines results in death. The acute and peracute forms majority affect piglets characterized by abdominal pain, depression and hemorrhagic diarrhoea. These clinical presentations lasts few hours upto 1 day after primary exposure to *Clostridium perfringens* type C [6].
The case fatality rates of *Clostridium perfringens* type C varies but the mortality rates of 100% in litters from non immune sows is not unusual and herd mortality could be more than 50% with higher herd immunity [7]. The present study focussed on molecular detection of *Clostridium perfringens* α and β toxin types from intestinal contents. The objective of the study is to assess which toxin genes are present in *Clostridium perfringens* strains isolated from young piglets with bloody diarrhoea using PCR specific for the α (cpa) and β (cpb) genes respectively.

Materials and Methods
Isolation of *Clostridium perfringens* from Intestine and Intestinal contents
Sampling
Intestines and Intestinal contents were collected aseptically in postmortem of piglets with bloody diarrhoea.

Isolation and Identification
Intestines with the contents were inoculated in to a tube of sterile freshly prepared nutrient broth as an enrichment medium followed by incubation aerobically at 37 °C for 24-48 hours. For isolation of *Clostridium perfringens*, a lapful of culture is streaked on to the 5% sheep blood agar. The plates are incubated aerobically at 37 °C for 24-48 hours.

Boiling and snapchill method of DNA extraction

100 µl of overnight grown culture was centrifuged at 5000 rpm for 10 minutes, supernatant was discarded and the pellet was resuspended in 100 µl of nuclease free water and kept in boiling water at 90 °C for 10 minutes. After heat treatment, the cell lysate was immediately kept on ice for 10 minutes followed by centrifugation at 10,000 rpm for 3 minutes. The supernatant containing the DNA was aliquoted in a sterile appendorf tube and stored at -20 °C until further use.

Amplification of 16SrRNA and toxin genes of *Clostridium perfringens*

The species specific primers targeting 16SrRNA gene, α and β toxin genes of Clostridium perfringens are examined by PCR (Table 1)

Table 1: Primers for the *Clostridium perfringens* 16srRNA and toxin genes (α and β-toxins)

S. No	Gene (Toxin)	Sequence (5' -3')	Product size(bp)	Reference
1	16srRNA	F-TAACCTGCCTCATAGAGT R- TTTCACATCCCACTTAATC	481	Reference [8]
2	Cpa (α-toxin)	F-GCTAATGTTACTGCCGTTGA R-CCTCTGATACATCGTGTAAG	324	Reference [9]
3	Cpb (β-toxin)	F-GCGAATATGCTGAATCATCTA R-GCAGGAACATAGTATATATCTTC	195	

Multiplex PCR assay for 16SrRNA and α-toxin genes of *Clostridium perfringens*

Multiplex PCR assay was performed in 25 µl reaction volume in thermal cycler. The reaction consisted of 3 µl of template, 12.5 µl of red dye 2xPCR master mix, 1 µl each of 10 pico moles of 16SrRNA and α-toxin gene primers, 5.5 µl of nuclease free water were used.

PCR assay for 16SrRNA and β-toxin genes of *Clostridium perfringens*

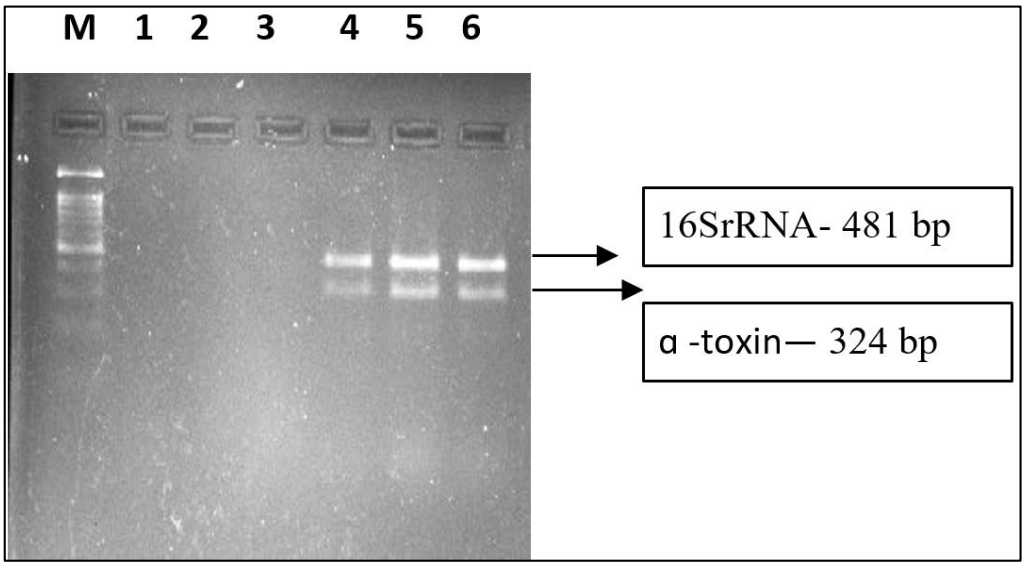
PCR assay was performed in 25 µl reaction volume in thermal cycler. The reaction consisted of 3 µl of template, 12.5 µl of red dye 2xPCR master mix, 1 µl each of 10 pico moles of 16SrRNA and β-toxin gene primers separately, 5.5 µl of nuclease free water were used.

Cyclical conditions for 16SrRNA and toxin genes based PCR

Initial denaturation at 95 ℃ for 15 minutes, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 49 °C for 90 sec, extension at 72 °C for 90 sec, final extension at 72 °C for 10 minutes. After amplification, 10 µl of amplified PCR products are mixed with 2.5 µl of the 6x loading dye. Samples are electrophoresed on 2% agarose gel.

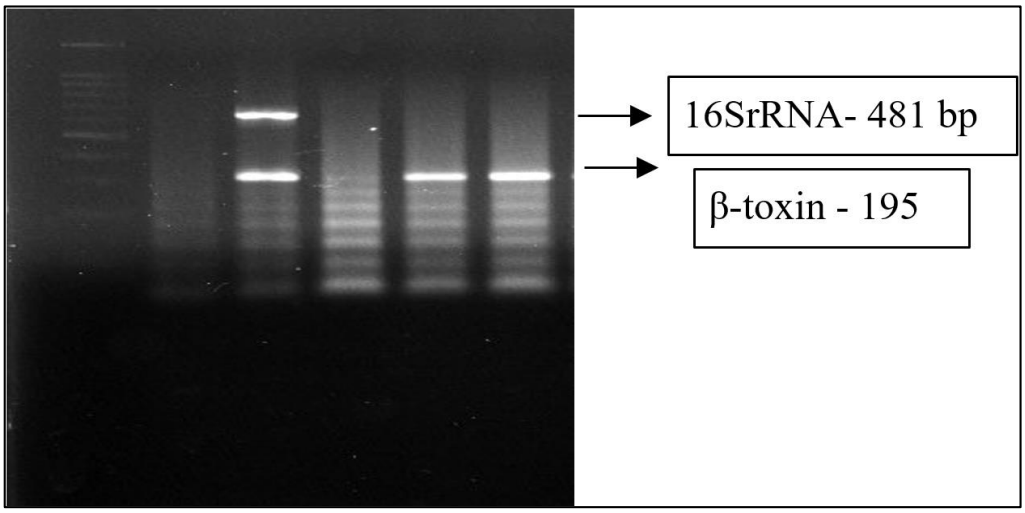
Results

Two isolates of *Clostridium perfringens* were obtained from intestines along with intestinal contents. The results of multiplex PCR showed that *Clostridium perfringens* strain was positive for the 16SrRNA gene with amplicon size of 481 bp and α-toxin gene (cpa) with amplicon size of 324 bp (Figure 1). PCR assay showed the presence of β-toxin gene (cpb) with amplicon size of 195 bp (Figure 2). These results indicate the presence of α-toxin and β-toxin which confirms the *Clostridium perfringens* type C isolates. Our study showed two isolates from the Intestinal contents of piglet detected with bloody diarrhea.



Lane-M-1500 bp DNA ladder
Lane 3: Negative control
Lane 4: Positive control of *Clostridium perfringens* with amplified 16SrRNA (481 bp) and α -toxin gene
Lane 5: *Clostridium perfringens* isolate 1 with amplified 16SrRNA& α -toxin genes
Lane 6: *Clostridium perfringens* isolate 2 with amplified 16SrRNA& α -toxin genes

Fig 1: Amplicon sizes of 16SrRNA and α-toxin genes



Lane-7-100 bp marker
Lane-8-Negative control
Lane-9-Positive control (*Clostridium perfringens* showing 16SrRNA and gene)
Lane -11- *Clostridium perfringens* isolate1 showing amplicon for β-toxin toxin gene
Lane-12- *Clostridium perfringens* isolate 2 showing amplicon for β-toxin gene

Fig 2: Amplicon sizes of 16SrRNA and β-toxin genes

Discussion

In the present study here, piglets with bloody diarrhoea or necrotic enteritis was examined for the presence of *Clostridium perfringens* type C based on the presence of toxin types of *Clostridium perfringens* by species specific PCR and toxin gene specific PCR. The results in the present study imply that the presence of cpa (α-toxin) and cpb (β-toxin) genes was confirmed as *Clostridium perfringens* and the presence of α-toxin and β-toxin genes belong to *Clostridium perfringens* type C. The diagnosis of *Clostridium perfringens* type C enteritis in pigs can be based on group of findings including history, clinical and pathological results, where as confirmation should be based on isolation of large numbers of *Clostridium perfringens* typeC and/ or cpb (β-toxin) gene detection from intestinal contents. The presence of *Clostridium perfringens* type C in the present study is in agreement with the findings of many authors who worked on clostridium enteric infections in pigs [10]. β-toxin is considered as the main virulent factor in *Clostridium perfringens* type C infections as it is very sensitive to the trypsin digestion [11]. The detection of main toxins in intestinal content and faeces, combination with clinical and pathological findings is diagnostic for *Clostridium perfringens* type C. Small numbers of organisms are usually isolated from the Intestines of healthy piglets, colonisation of intestines with large number of bacteria are isolated from diseased necrotic enteritis piglets.

Detection of cpb (β-toxin) gene is of great diagnostic value, as this toxin is not found in intestinal contents of healthy pigs. Strict hygiene practices should be followed in the non-vaccinated herds to reduce the newborn piglets mortality as this could be manifested as acute outbreak of necrotic enteritis. Immediate vaccination to sows in the herd also play major role in reducing mortality of the newborn piglets through sufficient amounts of clostridial antibodies. The development of vaccine that specifically protect the effects from β-toxin is of importance. It can be achieved by producing non-toxic form of vaccine either by chemical inactivation or by site directed mutagenesis. Diagnosis of necrotic enteritis in pigs with *Clostridium perfringens* type C could be based on detection of cpa and cpb toxins in faeces or in intestinal contents.

Conclusions

Two isolates of *Clostridium perfringens* type C were obtained with confirmation of α-toxin gene and β-toxin gene on Multiplex PCR.

Acknowledgement

Authors are thankful to the Vice-chancellor, Sri Venkateswara Veterinary University, Tirupati, AP, India for providing funding and infrastructure facility.

References

1. Fach P, Popoff MR. Detection of enterotoxigenic *Clostridium perfringens* in food and fecal samples with a duplex PCR and the slide latex agglutination test. Applied environmental microbiology. 1997;63:4232-4236.
2. Songer JG. Clostridial enteric diseases of domestic animals. Clinical Microbiology Reviews. 1996;9:216-234.
3. Rood JI, Adams V, Lacey J, Lyras D, McClane BA, Melville SB, *et al*. Expansion of the *Clostridium perfringens* toxin-based typing scheme. Anaerobe. 2018;53:5-10.
4. Songer JG, Meer RR. Genotyping of *Clostridium perfringens* by polymerase chain reaction is a useful adjunct to diagnosis of clostridial enteric disease in animals. Anaerobe. 1996;2:197-203.
5. Songer JG, *et al*. Clostridial enteric infections in pigs. Journal of Veterinary Diagnostic Investigation. 2005;17:528-536.
6. Diab SS. Diseases Produced by *Clostridium perfringens* Type C. In: Uzal, F.A., Songer, J.G., Prescott, J.F., Popoff, M.R. (Eds.), Clostridial Diseases of Animals. John Wiley & Sons, Inc, Hoboken, NJ, 2016, p. 143-155.
7. Hogh P. Necrotizing infectious enteritis in piglets, caused by *Clostridium perfringens* type C. II. Incidence and clinical features. Acta Veternaria Standinavica. 1967;8:301-323.
8. Tonooka T, Sakata S, Kitahara M, Hani M, Ishizeki S,

Takada M, *et al*. Detection and quantification of four species of the genus *Clostridium* in infant faces. Microbiology and Immunology. 2005;49(11):987-92.
9. Van Asten AJ, Allaart JG, Grone A, Houwers DJ. Application of PCR-based detection of *Clostridium perfringens* cpb-2 in feral samples. Veterinary Microbiology. 2008;129(1-2):215.
10. Songer JG, Uzal FA. Clostridial enteric infections in pigs. Journal of Veterinary Diagnostic Investigation. 2005;17:528-536.
11. Fischer DJ, Miyakawa FME, Sayeed S. Dissecting the lethality contributions of *Clostridium perfringens* type C toxins in mouse intravenous injection model. Infection and Immunology. 2006;74:5200-5210.