

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.606.309>

Molecular Detection and Pathology of Necrotic Enteritis Case from a Guinea Fowl

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ABSTRACT

Necrotic enteritis is an important bacterial disease of poultry caused by type A and type C of the enteric gram positive spore forming organism *Clostridium perfringens*. Necrotic enteritis suspected case was presented for necropsy from a dead guinea fowl. Gross examination showed ulcerative lesions in the intestine. Microscopically, necrosis and degeneration of the intestinal villi and mononuclear cell infiltration was noticed. Liver histopathology revealed that the mononuclear cells infiltration and perivascular cuffing in blood vessels. The causative organism was isolated from the intestinal contents under anaerobic condition. The collected sample was processed and growth was achieved on tryptose – sulphite – cycloserine (TSC) agar, blood agar, clostridial agar, perfringens agar. Typical *Clostridium perfringens* colonies were identified by grams staining and further confirmed by biochemical characterization. For molecular confirmation genomic DNA was isolated from *C. perfringens* and amplified using 16s rRNA universal primer. Multiplex PCR (mPCR) was carried out for toxin genotyping and the isolate was found positive for α toxin (cpa), a characteristic feature of *C. perfringens* type A. The present report emphasizes the molecular characterization and toxin genotyping as a rapid tool for detection of *C. perfringens* from suspected necrotic enteritis cases in poultry.

Keywords

Guinea fowl, mPCR, Necrotic enteritis, Toxin genotyping.

Article Info

Accepted:

29 May 2017

Available Online:

10 June 2017

Introduction

Necrotic enteritis is caused by an anaerobic gram positive, spore forming bacteria *Clostridium perfringens* type A and to a lesser extent the type C (Cooper and Songer, 2009). It is often found in the intestinal tract of healthy broiler chickens but it can cause necrotic enteritis (NE) in many species of poultry (Engstrom *et al.*, 2003). The disease primarily affects broilers 2-6 weeks of age and turkeys 7-12 weeks of age. However NE has been reported in Japanese quails and other fowls raised on litter.

The clinical illness is usually very short and often the only signs are severe depression followed quickly by a sudden increase in flock mortality.

The present study reports the isolation, identification, pathology and molecular detection of the causative organism *C. perfringens* causing necrotic enteritis in a guinea fowl, by 16s rRNA PCR followed by multiplex PCR for toxin genotyping.

Materials and Methods

An adult 8 week old dead female guinea fowl was necropsied at Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai and the sample was used for further processing. History revealed that the affected bird showed signs of in appetite, diarrhoea and debility. Samples were collected aseptically including intestinal contents, liver and intestinal scrappings for bacteriological examination and isolation of the organism. The tissues samples were processed for histopathological studies.

Isolation of *Clostridium perfringens* and PCR confirmation

For isolation of *Clostridium perfringens*, strict anaerobic conditions were maintained. Sterile saline was added to the intestinal contents of the sample and heated at 80°C for 20 min in water bath. Then the processed intestinal contents were inoculated into fluid thioglycollate broth, reinforced clostridial broth, Robertson cooked meat medium with brain heart infusion broth and incubated in anaerobic environment at 37 °C for 48h. About 100 µl of the culture suspension was placed into sterile Petri dish and overlaid with tryptose-sulphite-cycloserine (TSC) agar with selective supplement for perfringens (Himedia Pvt. Ltd., Mumbai) and without egg yolk emulsion for morphological characterization.

Further, samples from the broth also were streaked on the perfringens agar plate enchanted with egg yolk emulsion (EYA agar). To maintain anaerobic condition, plates were sparged with CO₂ gas and then sealed using parafilm. All the incubations were conducted in anaerobic chamber with anaerogas pack (Himedia Pvt. Ltd., Mumbai). DNA was extracted from the bacterial culture

using DNA extraction kit (Quiagen, USA) as per the manufacturer's instructions. The *C. perfringens* suspected DNA samples were amplified using 16s rRNA universal primer as follows 16s rRNA F- AGAGTTTGATYMTGGC and 16s rRNA R – GYTACCTTGTTACGACTT (Davies *et al.*, 1996).

For amplification, a 25 µl reaction mixture was prepared with 1.0 µl (10 picomole) of each primer, 12.5 µl of PCR master mix (Ampliqon), 2.0 µl of genomic DNA and 8.5 µl of nuclease free water. The amplified PCR product was confirmed by 1.5% agarose gel electrophoresis. Specific amplicon was purified using QIA quick Gel extraction kit and then sequenced in Leads India Pvt. Ltd., Kochi. Further, the sequences were aligned, assembled and analyzed by DNA Star Laser gene V. 7.0 software.

Toxin genotyping of the isolate was carried out by multiplex PCR targeting three major toxin genes *cpa*, *cpb*, *cpe* as described previously (Baums *et al.*, 2004).

Results and Discussion

Clostridium perfringens, the causative agent of necrotic enteritis was isolated and identified from a clinical case of guinea fowl. Gross examination revealed that pathological changes in the intestinal segments. The intestinal mucosa showed several oval to elongated necrotic to ulcerative lesions with raised hyperemic borders (Figure 1a). There are no significant pathological lesions in the other visceral organs were observed. Histopathological lesions showed the partial or complete damage of villi in the necrosis areas with mononuclear cell infiltration (Figure 1b) as reported earlier (van Immerseel *et al.*, 2009). Histopathology of liver showed congestion and mononuclear cell infiltration.

Bacterial colony morphology on blood agar showed a clear double zone of haemolysis that was similar to the findings of earlier report (Craven *et al.*, 2001; Malmarugan and Rajeswar, 2012). Yellowish grey colonies were observed in TSC agar with egg yolk supplementation (Figure 2a). The colonies on perfringens agar supplemented with egg yolk agar were identified by a typical opalescence around the colonies as in figure 2b. The lipoprotein component lecithovitellin in egg yolk agar is split by lecithinase into

phosphoryl choline and an insoluble diglyceride, which results in the formation of a precipitate in the medium. The precipitate occurs as a white halo, surrounding the colony producing it. The alpha toxin of *C. perfringens* produces lecithinase and hence white zone was indicative of *C. perfringens* strain containing alpha toxin. The findings of opalescence colonies in egg yolk suggestive of *Clostridium* and the observation was in agreement with the previous report (Barrow and Feltham, 1993).

Fig.1 Histopathological changes in (a) intestinal villi damage (b) mononuclear cell infiltration

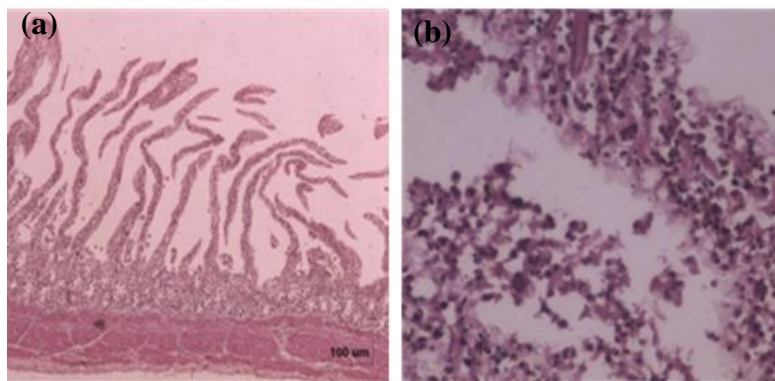


Fig.2 Growth pattern of *Clostridium perfringens* a. Yellowish grey colonies in TSC agar; b. Opalescent zone around the colonies due to lecithinase activity in egg yolk agar

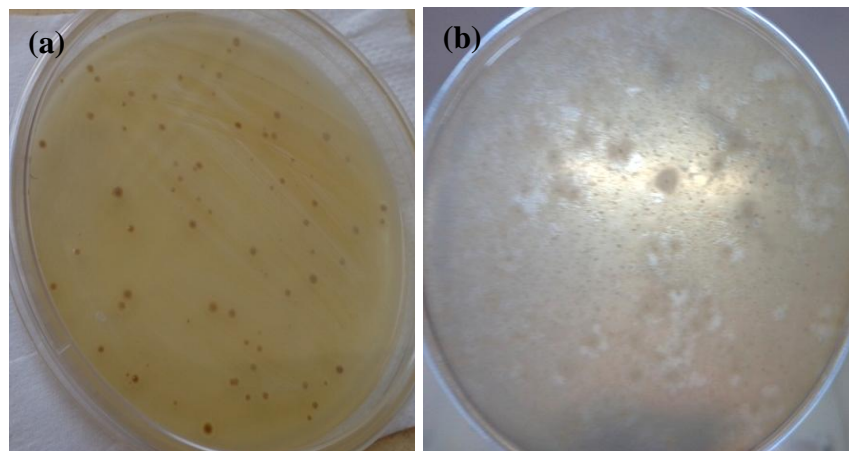


Fig.3 1.5% agarose gel showing 16srRNA gene amplification. Lane 1 – 100 bp ladder;
Lane 2 – 994 bp 16srRNA gene

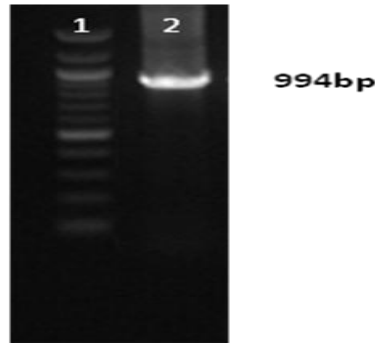
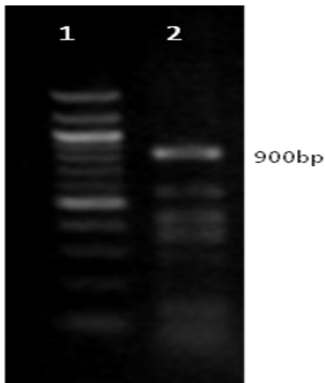


Fig.4 1.5% agarose gel showing amplification of *cpa* toxin gene. Lane 1- 100bp ladder;
Lane 2-900 bp *cpa* toxin gene amplicon



PCR amplicons of 994 bp were noticed on 1.5% agarose gel (Figure 3) and further confirmed by sequencing. Blast analysis showed that sequence had 93% homology when compared with the reference sequence of *C. perfringens* (AB566417.1) available in the genbank.

Multiplex PCR for toxin genotyping of *C. perfringens* was positive for 900 bp *cpa* toxin gene PCR amplicon (Figure 4), a feature indicative of *C. perfringens* type A isolate. It is well documented that α toxin producing *C. perfringens* is more common in intestinal microbiota of diseased chicken.

And our result suggested that the prevalence of type A, *C. perfringens* in the intestine of poultry species with necrotic enteritis

harboring the α -toxin gene as predominant toxinotype similar to the previous reports of Engstrom *et al.*, (2003) and Thomas *et al.*, (2014) in broiler and Kadakanath fowl respectively.

This paper reports the isolation and identification of *C. perfringens*, causing necrotic enteritis in a guinea fowl. Molecular detection, characterization by 16s rRNA gene sequencing and toxin genotyping by multiplex PCR were utilized for rapid detection and confirmation of *C. perfringens* from the suspected case.

References

Barrow, G. I., and Feltham, R. K. A. 1993. Cowans and Steel's Manual for

- identification of medical bacteria. 3rd ed., Cambridge University Press, pp.331.
- Baums, C. G., U. Schotte, G. Amsberg and Goethe, R. 2004. Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. *Veterinary Microbiology*. 100: 11-16.
- Cooper, K. K., and Songer, J. G. 2009. Necrotic enteritis in chickens: A paradigm of enteric infection by *Clostridium perfringens* type A. *Anaerobe*. 15: 55-60.
- Craven, S. E., N. J. Stern, J. S. Bailey and Cox, N. A. 2001. Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. *Avian Diseases*. 45: 887-896.
- Davies, C., V. Ramakrishnan and White, S. W. 1996. Structural Evidence for specific S8-RNA and S8 - protein interactions within the 30S ribosomal subunit: ribosomal protein S8 from *Bacillus stearothermophilus* at 1.9 Å resolution. *Structure*. 4: 1093-1104.
- Engstrom, B. E., C. Fermer, A. Lindberg, E. Saarinen, V. Baverud and Gunnarsson, A. 2003. Molecular typing of isolates of *Clostridium perfringens* from healthy and diseased poultry. *Veterinary Microbiology*. 94: 225-235.
- Malmarugan, S., and Rajeswar, J. 2012. Alpha toxin specific PCR for detection of toxigenic strains of *Clostridium perfringens* in Poultry. *Veterinary World*. 5(6): 365-368.
- Thomas, P., T. R. Arun, K. Karthik, P. V. Berin, M. Ashok Kumar, Neethu Singh, J. Usharani, M. Palanivelu, S. K. Gupta, K. Dhama and Viswas, K. N. 2014. Molecular characterization and toxinotyping of a *Clostridium perfringens* isolate from a case of necrotic enteritis in Indian Kadaknath fowl. *Asian Journal of Animal and Veterinary Advances*. 9: 385-394.
- Van Immerseel, F., J. I. Rood, R. J. Moore and Titball R. W. 2009. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. *Trends Microbiology*. 17: 32-36.

How to cite this article:

Rathnapraba, S., Kannabhiran, P. Kanagaraju, K. Vijayarani and Saranya, S. 2017. Molecular Detection and Pathology of Necrotic Enteritis Case from a Guinea Fowl. *Int.J.Curr.Microbiol.App.Sci*. 6(6): 2589-2593. doi: <https://doi.org/10.20546/ijcmas.2017.606.309>