



FIRST RECORD OF NECROTIC ENTERITIS CAUSED BY *Clostridium perfringens* IN CHICKENS FROM CHATTISGARH (INDIA)

Chandrabhas Sannat*, R.K. Shende, V. Dilliwar and S.D. Hirpurkar

Department of Veterinary Microbiology, College of Veterinary Science & Animal Husbandry,
Anjora, Durg - 491 001 Chhattisgarh (India)

*e mail: csannat@rediffmail.com

(Received 11 April, 2014; accepted 25 August, 2014)

The outbreak of several devastating diseases is one of the major constraints causing economic loss so discouraging poultry rearing in India. Intestinal diseases are an important concern for modern poultry industry because of low productivity, increased mortality and the associated contamination of poultry products with pathogenic bacteria and their toxins. Necrotic enteritis is one of the world's most common diseases which cause mortality up to 10-40% (McDevit *et al.*, 2006). Necrotic enteritis caused by *Clostridium perfringens* in chickens is a risk to public health through food chain (Van Immerseel *et al.*, 2004). Necrotic enteritis in chicken due to *C. perfringens* has not earlier been reported from Chhattisgarh (India).

The birds for present study were reared under Poultry Seed Project at College of Veterinary Science & AH, Anjora, Durg (CG) during March, 2013. Thirty five layer chicken of Vanraja breed died in a flock of 400 birds within 5 days. Post-mortem examination revealed extensive necrotic lesions in intestine of birds suggestive of necrotic enteritis (Cooper and Songer, 2010; Fayez *et al.*, 2013). The jejunal contents, collected aseptically in brain heart infusion (BHI) broth, were processed for pathogen isolation as per Eyre (2009). The samples were inoculated into BHI broth and 2-3 mL olive oil poured over the surface of culture broth to create anaerobic conditions. Broth culture was kept in candle jar and incubated for 24 hr at 37°C. BHI broth culture was further inoculated in anaerobic media i.e. Robertson cooked meat medium (RCMM) for selective enrichment (Dahiya *et al.*, 2007). Culture smears from RCMM showing the presence of clostridia organisms were inoculated on 5% sheep blood agar and egg yolk agar and incubated anaerobically in candle jar at 37°C for 24 hr and 5 days, respectively. The colonies of each isolate were inoculated by stabbing into triple sugar iron (TSI) agar slants. Besides, morphological characterization, the motility of organisms was judged by hanging drop technique and biochemical tests viz., catalase, methyl red (MR), Voges-Proskauer (VP) and indole tests were performed.

The outbreak of necrotic enteritis in chickens was noticed with 8% mortality. Earlier, the outbreaks of necrotic enteritis have been reported from Bangladesh (Miah *et al.*, 2011), Australia (Nairn and Bamford, 1967), USA (Craven *et al.*, 2001) and some states of India (Sawale *et al.*, 2010; Shanmugasamy *et al.*, 2012). The impression smear of 10 samples revealed the presence of numerous large thick non-motile Gram positive rods. The organisms were sporulating bacilli, produced double zone of hemolysis on blood agar, opalescent growth on egg yolk agar and yellowish and black precipitate over surface (H₂S positive) of TSI agar. The catalase, indole, MR and VP tests were negative. However, the culture smears from only three RCMM isolates confirmed the morphology of *Clostridium* sp. (Rhodehmel *et al.*, 1998; Shamimuzzaman, 1999). Cultural characteristics and biochemical pattern of these isolates were similar to *C. perfringens* (Rhodehmel *et al.*, 1998). The necrotic enteritis in chicken has earlier been reported from Maharashtra (Sawale *et al.*, 2010), Tamilnadu (Shanmugasamy *et al.*, 2012) and Meghalaya (Das *et al.*, 2008) but is *hitherto*

unreported from Chhattisgarh. The present report, therefore, is the first record of necrotic enteritis caused by *C. perfringens* in chickens from Chhattisgarh (India).

-
- Cooper, K.K and Songer, J.G. 2010. Virulence of *Clostridium perfringens* in an experimental model of poultry necrotic enteritis. *Veterinary Microbiology*, **142**: 323-328.
- Craven, S.E., Stern, N.J., Bailey, J.S and Cox, N.A.2001. Incidence of *Clostridium perfringens* in broiler chickens and their environment during production and processing. *Avian Diseases*, **45**: 887-896.
- Dahiya, J.P., Hoehler, D., Van Kessel, A.G and Drew, M.D. 2007. Dietary encapsulated glycine influences *Clostridium perfringens* and Lactobacilli growth in the gastrointestinal tract of broiler chickens. *Journal of Nutrition*, **137**: 1408-1414.
- Das, A., Mazumder, Y., Dutta, B.K., Shome, B.R., Bujarburuah, K.M and Kumar, A. 2008. *Clostridium perfringens* type A from broiler chicken with necrotic enteritis. *International Journal of Poultry Science*, **7**: 601-609.
- Eyre, J.W.H. 2009. *The Project Gutenberg eBook of The Elements of Bacteriological Technique: A Laboratory Guide for Medical, Dental and Technical Students* (2nd edn.). eBook #27713 ISO-8859-1.
- Fayez, M.M., Abdelaziz, A.M. and Al-Musallam, A. 2013.Characterization of toxigenic strains of *Clostridium perfringens* type A from broiler chicken with necrotic enteritis. *International Journal of Advanced Research*, **1**: 321-327.
- McDevit, R.M., Brooker, J.D., Acamovic, T. and Sparks, N.H.C. 2006. Necrotic enteritis; continuing challenge for the poultry industry. *World's Poultry Science Journal*, **62**: 221- 247.
- Miah, M.S., Asaduzzaman, M., Sufian, M.A. and Hosain, M.M. 2011. Isolation of *Clostridium perfringens*, causal agents of necrotic enteritis in chickens. *Journal of the Bangladesh Agricultural University*, **9**: 97-102.
- Nairn, M.E. and Bamford, V.W. 1967. Necrotic enteritis of broiler chickens in Western Australia. *Australian Veterinary Journal*, **43**: 49-54.
- Rhodehamel, G., Bruce, H.L., Hunter, D.B., Parreira, V.R., Kulkarni, R.R., Jiang, Y.F., Prescott, J.F. and Boerlin, P. 1998. *Multilocus Sequence Typing Analysis of Clostridium perfringens Isolates from Necrotic Enteritis Outbreaks in Broiler Chicken Populations*. Department of Pathobiology, Ontario Veterinary College, University of Guelph, Ontario, Canada.
- Sawale, G.K., Mane, D.V., Shelke, V.M. and Bharkad, G.P.2010. Occurrence of necrotic enteritis in commercial layer chickens raised in cages. *Indian Journal of Veterinary Pathology*, **34**: 138-140.
- Shamimuzzaman, A.H.M. 1999. *Characterization of Clostridium perfringens Isolated from Goat and its Antibiotic Sensitivity*. M.Sc. thesis. Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.
- Shanmugasamy, M., Adhimoolam, B. and Natarajan, D. 2012. Necrotic enteritis in broiler and layer farms in Tamilnadu. *International Journal for Agro-Veterinary and Medical Sciences*, **6**: 241-249.
- Van Immerseel, F., De Buck, J., Pasmam, F., Huyghebaert, G., Haesebrouck, F. and Ductelle, R. 2004. *Clostridium perfringens* in poultry: An emerging threat for animal and public health. *Avian Pathology*, **33**: 537-549.