

## STUDY ON CLOSTRIDIUM PERFRINGENS TYPE A INFECTION IN BROILERS OF WEST BENGAL, INDIA

M. Sarkar<sup>1</sup>, J. P. Ray<sup>1</sup>, S. K. Mukhopadhyay<sup>2\*</sup>, D. Niyogi<sup>3</sup>, S. Ganguly<sup>4</sup>

<sup>1</sup>Dept. of Veterinary Microbiology, Faculty of Veterinary & Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, INDIA

<sup>2</sup>Dept. of Veterinary Pathology, Faculty of Veterinary & Animal Sciences, West Bengal University of animal and Fishery Sciences, Kolkata - 700 037, INDIA

<sup>3</sup>Dept. of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Narendra Deva University of Agriculture and Technology, Faizabad - 224 229, INDIA

<sup>4</sup>AICRP-PHT (ICAR), Department of Fish Processing Technology, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 094, WB, INDIA

### ABSTRACT

This study was conducted to report the incidence of *Clostridium perfringens* Type A in the broiler chicken, poultry feed and farm environment and also to find out the most effective antibiotic on the isolates. A total of 900 samples comprising of 400 samples from dead broiler birds, 150 samples from compound poultry feed, 150 samples of poultry feed components of animal source origin and 200 samples of farm environment (litter, wall swab and water) were collected from different poultry farms of North 24 Parganas, Kolkata, Howrah and Nadia districts of West Bengal (India) and were processed for the isolation of the organism and finally, the isolates were tested for antibiotic sensitivity. The result showed that the incidence rate was higher in compounded poultry feed (59.33%) followed by poultry feed components of animal origin (54%), farm environment (53%) and dead broiler birds (50.25%) indicating the chance of establishing infection among broiler birds clinically or sub-clinically. The antibiotic sensitivity test revealed that Penicillin-G is the most effective drug against the isolates of *C. perfringens* Type A, whereas gentamicin, streptomycin, kanamycin and tetracycline were found ineffective suggesting the use of antibiotics after proper investigation of drug resistance.

Received on: 18<sup>th</sup>-March-2013

Revised on: 13<sup>th</sup>-April-2013

Accepted on: 25<sup>th</sup>- April-2013

Published on: 15<sup>th</sup>-July-2013

#### KEY WORDS

Antibiogram, Broiler chicken, *Clostridium perfringens* Type A, Incidence

\*Corresponding author: Email: [skmpath@gmail.com](mailto:skmpath@gmail.com); Tel: +91 9433036799

### [I] INTRODUCTION

*Clostridium perfringens* Type A is a bacterial pathogen causing necrotic enteritis in broiler birds and is responsible for causing both visible and invisible economic losses through mortality, morbidity, weight loss, low feed conversion ratio and poor performance. *C. perfringens* Type A is also associated with other poultry diseases including avian malignant gas edema [1], gizzard erosion [2] and gangrenous dermatitis [3]. A high number of *C. perfringens* Type A in the intestinal tract and associated necrotic lesion have been detected world wide in poultry flocks that suffer from necrotic enteritis [4, 5]. To combat the disease as well as to minimize the losses due to this bacterial infection in poultry, proper investigation, and identification of the organism and antibiogram studies are very essential. Keeping in view these fact, the present work was planned to predict the prevalence of *C. perfringens* Type A in poultry feed and farm environment and also to find out the most suitable antibiotics to be effective on the isolates.

### [II] MATERIALS AND METHODS

A total number of 900 samples amongst which 400 samples from dead poultry birds of 2-6 weeks age having the history of enteritis, diarrhea and low feed conversion ratio and frequent mortality; 150 samples from compound poultry feed i.e. broiler starter, finisher, developer pellet feed and poultry feed containing vegetable protein; 150 samples of poultry feed components of animal source origin like fish meal, meat cum bone meal, meat meal and blood meal and 200 samples of litter, wall swab and water were collected from different poultry farms of North 24 Parganas, Kolkata, Howrah and Nadia districts of West Bengal (India). These samples were processed for the isolation of *C. perfringens* Type A as per the method described by Cruickshank et al. [6] and Balows et al. [7]. Cooked meat medium (RC medium) and tryptose sulfite cycloserine (TSC) agar, *Perfringens* agar were used for isolation and identification on the basis of cultural, morphological and biochemical characteristics. *Perfringens* agar was used for the susceptibility test with 24 h incubation period [8]. Overall, randomly 100 isolates of *C. perfringens* Type A were tested for antibiotic sensitivity against antibiotics viz., gentamycin, kanamycin, neomycin, streptomycin, tetracycline, amikacin, cotrimoxazole, cloxacillin, amoxicillin, ampicillin, lincomycin, chloramphenicol, metronidazole, erythromycin and penicillin-G using the disc diffusion method [8].

### [III] RESULTS

Overall, 477 (53%) isolates of *Clostridium perfringens* Type A were recovered from the total 900 samples of which 201 (50.25%) from dead broiler birds, 89 (59.33%) from compounded poultry feed, 81 (54%) from poultry feed components of animal origin and 106 (53%) from farm environment. The percentage of incidence / prevalence was higher in compounded poultry feed followed by poultry feed components of animal origin, farm environment and dead broiler birds [Table -1]. Out of 400 samples (intestine and liver) of dead broiler birds, 201 (50.25%) isolates of *C. perfringens* Type A were recovered. In compounded poultry feed, out of 50 samples of broiler starter, 50 samples of broiler finisher, 25 samples of developer pellet and 25 samples of poultry feed containing vegetable protein yielded 36 (72%), 30

(60%), 13 (52%) and 10 (40%) isolates of *C. perfringens* Type A respectively. Among the farm environmental samples in the form of litter (100), wall swab (40) and water (60), isolates of *C. perfringens* Type A was found in 65 (65%), 21(52.5%) and 20(33.33%) samples respectively.

The results of antimicrobial sensitivity test revealed that 78% isolates were sensitive to penicillin-G followed by chloramphenicol (76%), lincomycin (73%), ampicillin (67%), metronidazole (60%), amoxicillin (57%), cloxacillin (5%), erythromycin (50%), cotrimoxazole (40%), neomycin (20%) and amikacin (8%). some isolates were intermediately sensitive to erythromycin (15%), penicillin-G (12%), neomycin (10%), chloramphenicol (9%), lincomycin (7%), kanamycin (5%) and amikacin (2%). All the isolates (100%) were found resistant to gentamicin, streptomycin and tetracycline.

Table-1: Incidence of *Clostridium perfringens* Type A in various samples

| Source                          | Sample                                    | Number of samples collected | No. of samples positive for <i>C. perfringens</i> Type A | Sample wise of incidence percentage (%) | Total number of samples positive for <i>C. perfringens</i> Type A from each source | Percentage of incidence (%) from each source |
|---------------------------------|-------------------------------------------|-----------------------------|----------------------------------------------------------|-----------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------|
| Dead poultry birds              | Intestine, liver                          | 400                         | 201                                                      | 50.25                                   | 201                                                                                | 50.25                                        |
| Compounded feed                 | Broiler starter                           | 50                          | 36                                                       | 72                                      | 89                                                                                 | 59.33                                        |
|                                 | Broiler Finisher                          | 50                          | 30                                                       | 60                                      |                                                                                    |                                              |
|                                 | Developer pellet                          | 25                          | 13                                                       | 52                                      |                                                                                    |                                              |
|                                 | Poultry feed containing vegetable protein | 25                          | 10                                                       | 40                                      |                                                                                    |                                              |
| Poultry feed from animal origin | Fish meal                                 | 50                          | 27                                                       | 54                                      | 81                                                                                 | 54                                           |
|                                 | Meat cum bone meal                        | 50                          | 26                                                       | 52                                      |                                                                                    |                                              |
|                                 | Meat meal                                 | 35                          | 20                                                       | 57                                      |                                                                                    |                                              |
|                                 | Blood meal                                | 15                          | 8                                                        | 53.33                                   |                                                                                    |                                              |
| Farm environment                | Litter                                    | 100                         | 65                                                       | 65                                      | 106                                                                                | 53                                           |
|                                 | Wall swab                                 | 40                          | 21                                                       | 52.5                                    |                                                                                    |                                              |
|                                 | Water                                     | 60                          | 20                                                       | 33.33                                   |                                                                                    |                                              |
| <b>TOTAL</b>                    |                                           | <b>900</b>                  | <b>477</b>                                               | <b>53</b>                               | <b>477</b>                                                                         | <b>53</b>                                    |

### [IV] DISCUSSION

The finding in the present study fully corroborates with the findings of Hussein and Mustafa [9]. The incidence of *C. perfringens* Type A in the environmental samples in the present study was close to the observation of Carven [10]. The results depicted in the present study were similar to the findings of Kohler [11] who also isolated 72 isolates of *Clostridium perfringens* from 102 compounded feed samples. In poultry feed components of animal origin, 50 samples of fish meal, 50 samples of meat cum bone meal, 35 samples of meat meal and 15 samples of blood meal yielded 27 (54%), 26 (52%), 20 (57%) and 8 (53.33%) isolates of *C. perfringens* Type A respectively.

Similar findings were also reported by Komnenov et al. [12] and Pupavac and Lalic [13].

The present findings supported the observation of Hussein and Mustafa [9] and Ibrahim et al. [14]. Higher sensitivity to penicillin-G and complete resistance to Streptomycin and Tetracycline was also recorded earlier [15]. However, there are reports of sensitivity of *C. perfringens* type-A to tetracycline [16].

100% resistance of the isolates to the tetracycline observed in the present study may be attributed to difference in isolates of *C. perfringens* Type A as determinant of resistance is frequently

transferable through plasmid salt transfer and plasmid mobilization [17]. Das et al. [18] studied the drug resistance pattern of *C. perfringens* type-A isolated from dead broiler and layer birds in Assam and West Bengal and recorded 100% efficacy of benzyl penicillin, chloramphenicol, erythromycin, lincomycin and metronidazole against the isolates. This variation of the sensitivity of the antimicrobial agents may be due to different antigenic constituents of *C. perfringens* Type A. Again, such differences in sensitivity may be attributed to the frequent use of antimicrobials for treatment and / or as a growth promoter in the feed.

## [VI] CONCLUSION

*Clostridium perfringens* Type A was found to be wide spread in the poultry farm environment including poultry feed and among broiler birds infected clinically or sub-clinically in West Bengal, India. It is also evident that Penicillin-G can be the drug of choice in *C. perfringens* Type A infection in broiler birds whereas gentamicin, streptomycin, kanamycin and tetracycline were found ineffective. So, indiscriminate and injudicious use of antibiotics/antimicrobial agents should be restricted and they must be used after a proper investigation of drug resistance.

*C. perfringens* is frequently found in the intestinal tract of healthy poultry. Isolation of *C. perfringens* may not show active infection sometimes Necrotic enteritis B like toxin (NetB) is critical virulence factor in the pathogenesis of necrotic enteritis in broilers. Virulence factors in the isolates have not been investigated in the present study.

## CONFLICT OF INTERESTS

The authors declare no competing interest in relation to work.

## FINANCIAL DISCLOSURE

Nil

## ACKNOWLEDGEMENTS

The authors are thankful to Hon'ble Vice-Chancellor, West Bengal University of Animal and Fishery Sciences for providing all the necessary facilities to carry out this original research work.

## REFERENCES

- [1] Hofcare CL, Frech JD, Page RK, Fletcher OJ. [1986] Subcutaneous Clostridial infection in broilers. *Avian Dis* 30: 620-622.
- [2] Fossum O, Sandstedt K, Engstrom BE. [1988] Gizzard erosions as a cause of mortality in white leghorn chickens. *Avian Pathol* 17: 519-525.
- [3] Gerdon D. [1973] Effects of mixed Clostridial bacteria in incidence of gangrenous dermatitis. *Avian Dis* 17: 205-206.
- [4] Long JR, Petti JR, Barnum DA. [1974] Necrotic enteritis in broiler chickens. II. Pathology and proposed pathogenesis. *Can J Comp Med* 38: 467-475.
- [5] Tasai SS, Tung MC. [1981] An outbreak of necrotic enteritis in broiler chickens. *J Chinese Soc Vet Sci* 7: 13-17.
- [6] Cruickshank R, Duguid JP, Marimon BP, Swain RHA. [1980] Medical Microbiology, 12th ed. Vol.-II. Churchill Livingstone Edinburgh, London, pp. 108-147.
- [7] Balows A, Hauster Jr, Herrmann KL, Shadmony HJ. [1991] Manual of Clinical Microbiology, 5th ed. American Society for Microbiology. Washington DC 28: 222-237.
- [8] Francisco L, Sapico, Yung-Yuan Kwok, Vera L. Sutter, and Sydney M Finegold. [1972] Standardized Antimicrobial Disc Susceptibility Testing of Anaerobic Bacteria: In Vitro Susceptibility of Clostridium perfringens to Nine Antibiotics. *Antimicrob Agents Chemother* 2(4): 320-325.
- [9] Hussein SZ, Mustafa FA. [1999] Necrotic enteritis in broiler chickens in Assiut Governorate. *Assiut Vet Med J* 41(82): 239-246.
- [10] Craven SE. [1990] The effect of pH of the sporulation environment on the heat resistance of Clostridium perfringens. *Curr Microbiol* 20(4): 223-237.
- [11] Kohler B, Kolbach S, Meine J. [1974] Studies on necrotic enteritis of fowl. II. Microbiological aspect. *Monatshefte fur Veterinormedizin* 29(10): 385-391.
- [12] Komnenov V, Velhner M and Katinka M. [1981] Importance of feed in the occurrence of Clostridial infections in poultry. *Veterinarski-Glasnik* 35(3): 245-248.
- [13] Pupavac V, Lalic M. [1990] Effect of bacterial contamination of animal feed on the health of animals. *Krimva* 32: 215-220
- [14] Ibrahim RS, Monaze IM, Soliman AM. [2001] Epidemiological studies on intestinal clostridial infection in broiler chickens in Assiut and EI Menia Governorates. *Assiut Vet Med J* 44(88): 291-305
- [15] Konado F. [1988] In vitro lecithinase activity to 22 antimicrobial agents of Clostridium perfringens isolated from necrotic enteritis of broiler chickens. *Res Vet Sci* 45(3): 337-340
- [16] Secasiu V. [1995] Chemo-sensitivity of Clostridium perfringens strains isolated from poultry. *Revista. Romana. De Medicina. Veterinara* 5(1): 31-37.
- [17] Sebald M. [1994] Genetic basis for antibiotic resistance in anaerobes. *Clin Infect Dis* 18(4): 297-304 (1994)
- [18] Das BC, Dutta GN, Devriese LA, Phukan A. [1997] Antimicrobial susceptibility of Clostridium Perfringens isolated from necrotic enteritis cases of poultry. *Indian Vet J* 74(7): 553-555.