NECROTIC ENTERITIS IN CHICKENS: PATHOLOGICAL, BACTERIOLOGICAL AND THERAPEUTICAL INVESTIGATION

M. N. ISLAM[1], S. M. H. RASHID[1], M. S. B. JULI[2], M. F. HOQUE[2] AND M. R. AKTER[3]

[1]Department of Pathology and Parasitology, [2]Department of Medicine, Surgery and Obstetrics, and [3]Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.

Accepted for publication: 20 May 2009

ABSTRACT

Islam, M. N., Radhid, S.M.H., Juli, M.S.B., Hoque, M.F., and Akter, M.R. 2009. *Necrotic enteritis in chickens: pathological, bacteriological and therapeutical investigation.* Int. J. Sustain. Crop Prod. 4(3):1-7.

Necrotic enteritis (NE) in commercial chickens was investigated around Hajee Mohammad Danesh Science and Technology University at Dinajpur of Bangladesh during 2007 to 2008 and diagnosed based on pathological, bacteriological and therapeutical findings. The disease is commonly found in commercial poultry farms and causes moderate to severe economic loss to the small scale poultry farmers by a remarkable mortality of the birds and their reduced weight gain. Among the 17 NE incidences, 9 in broiler, 5 in layer and 3 in cockerel flocks were detected during the course of the study. The number of the birds/farms was variable and they were reared on litter. The clinical signs as well as necropsy findings were noted during the physical visit of the farms and in laboratory when the birds were submitted. The recording of morbidity and mortality rates, bacteriological identification of the causative organisms, histopathological study of the preserved intestinal samples and therapeutic managements of the affected flocks without performing drug sensitivity were also done. The clinically affected birds generally showed moderate to severe depression, diarrhoea and death. The morbidity rate was around 100% but mortality rate was variable. At necropsy the birds were good bodily condition and severely dehydrated. Markedly thickened mucosa revealed yellow-brownish diphtheritic membrane, haemorrhages occasionally and ballooning of intestine with gas were the major gross morbid lesions. Clostridial organisms were isolated and identified on bacteriological examinations. Better therapeutic responses to oxytetracycline and tiamulin hydrogen fumerate along with carbon tetracycline were recorded.

Keywords: Necrotic enteritis, commercial chicken, microscopic examination

INTRODUCTION

Clostridium perfringens (CP) is a common inhabitant of the chicken intestinal tract, with no apparent impact on the host (Dutto and Devriese, 1980; Niilo, 1980; Benno *et al.*, 1988; Ficken and Wages, 1997). *Clostridium perfringens* is the causative agent of NE (Long, 1973; Tsai and Tung, 1981) and the contaminated feed and litter are the common sources of CP infection. CP causes a spectrum of illness including subclinical infection (Stutz *et al.*, 1983), mild clinical infection including diarrhoea (Hofshagen and Kaldhusdal, 1992), and the classical form of NE (Shane *et al.*, 1985). Outbreaks of NE have been reported worldwide (Frame and Bickford, 1986). A spectrum of clinical expression is well recognized for a variety of enteric pathogens like *E. coli* infection in clinically healthy individuals (Wilson *et al.*, 1996) and avian coccidiosis (McDougald, 2003).

Coccidiosis is a predisposing factor for NE (Shane *et al.*, 1985; Frame and Bickford, 1986). Mucosal damage by *Eimeria spp.* provides a surface for CP to proliferate (Al-Sheikhly and Al-Saieg, 1980). Lesions produced by *Eimeria brunetti* can be similar to those in necrotic enteritis, but uncomplicated coccidiosis is seldom as acute or severe. Ulcerative enteritis can resemble necrotic enteritis clinically, but the intestinal lesions are usually focal and located in the ileum, caeca, and rectum (Fraser, *et al.*, 1998).

It is hypothesized that dietary changes may alter the intestinal micro-environment in a manner which promotes clostridial overgrowth or stimulates toxin production in the intestinal lumen (Kaldhusdal and Skjerve, 1996). The disease is treated with various antibiotics like penicillin, erythromycin, and tetracycline, bacitracin, lincomycin, tylosin mentioned by some authors (Charlton, 2000; Fraser *et al.*, 1998; Wilson, *et al.*, 1996). NE is undoubtedly an economically important disease and the present study was carried out to investigate NE in commercial chickens based on clinical, pathological, bacteriological and therapeutical findings.

MATERIALS AND METHODS

Experimental chickens/Cases history

This study was conducted to diagnose NE in commercial chickens during the physical visit of the farms and when submitted to Pathology Laboratory of Hajee Mohammad Danesh Science Technology University at Dinajpur of Bangladesh.

A total of 17 incidences of NE, 9 in broiler, 5 in layer and 3 in cockerel flocks were detected during 2007 to 2008. The flock history including types of birds, total incidences, and population of birds per flock, rearing system, and age of birds, morbidity, mortality as well as the number of birds examined was presented (Table 1). The morbidity and mortality rates were determined from the farm records. The clinical signs of the affected flocks were recorded during the physical visit of the farm and the farmer's complaints in connection to it was also considered and noted.

Table 1. Clinical history of the different flocks examined

Type of birds	Age of birds (Days)	Previous disease history	Population of Birds/flock	No. of bird died due to NE	Morbidity rate (%)	Mortality rate (%)	Bird(s) examined at necropsy
Broiler	23	Coccidiosis	345	29	Around 100	8.41	**2 – 5 birds/flock**
	27	–	515	31	As above	6.00	
	24	–	412	27	As above	6.55	
	32	Coccidiosis	619	53	As above	8.56	
	21	–	326	31	As above	9.51	
	21	Gumboro disease	832	28	As above	3.37	
	20	–	246	19	As above	7.72	
	25	Gumboro disease and coccidiosis	377	37	As above	9.81	
	29	Gumboro disease	484	39	As above	8.06	
Layer	112	Gumboro disease and coccidiosis	450	27	Around 30	6.00	
	78	–	475	19	Around 50	4.00	
	94	–	550	11	Around 40	2.00	
	46	Coccidiosis	1140	57	Around 15	5.00	
	63	Gumboro disease	243	17	Around 100	7.00	
Cockerel	49	Gumboro disease and coccidiosis	344	55	As above	15.99	
	67	Gumboro disease	529	37	Around 50	6.99	
	76	Gumboro disease and coccidiosis	938	122	As above	13.00	

NECROPSY AND HISTOPATHOLOGICAL EXAMINATION

Both sick and dead birds submitted for diagnosis and treatment were examined systematically at necropsy following standard procedure (Charlton, 2000). The birds were also brought to laboratory during physical visit. The clinical history and signs were carefully considered before the attempt of postmortem examination. The physical appearances of the carcasses and the visible gross morbid lesions of the intestines were recorded.

The tissue samples were collected during the course of necropsy and preserved at 10% formalin solution as soon as possible to avoid the alteration of the tissues through autolysis. The autolysed tissues were avoided for histopathological examination. The fixed samples were processed, embedded in paraffin, sectioned and stained with haematoxylin and eosin following a well recommended procedure (Luna, 1968). The characteristic histopathological lesions were observed under light microscope and recorded.

BACTERIOLOGICAL FINDINGS

The intestines containing lesions were collected during necropsy and brought to the bacteriological examinations with necessary precaution. The Gram stained impression smears prepared from the collected samples were made with a standard procedure (McLeod *et al.*, 1981) to demonstrate the cellular morphology and arrangement of the bacteria.

Primary isolation of the bacteria in culture from the collected tissue samples were made by standard routine laboratory methods (Benson, 1984) by using blood agar media. The organisms were cultured on blood agar plates incubated anaerobically at 37ºC and the colony characteristics were recorded. Identification of the bacteria was determined performing biochemical reactions in differential media (glucose, maltose, lactose, sucrose, and mannitol) and the results were noted. Typification of the organisms was not done. Faeces and tissue scraps were microscopically examined in different magnifications to identify coccidia, if any, and differentiate NE from coccidiosis.

THERAPEUTIC FINDINGS

The affected flocks were treated primarily based on the necropsy findings. Different commercially available antibiotics: oxytetracycline (Renamycin Powder, Renata Animal Health, Bangladesh @ 3 gram per 5 litre of drinking water daily for 5 days), doxycycline (Doxivet, Renata Animal Health, Bangladesh @ 1gram per 2 litre of drinking water daily for 5 days), trimethoprim-sulphadiazine combination (Sulphatrim suspension, Rampart Power, Bangladesh @ 1ml per 5 litre of drinking water daily for 5 days), tiamulin hydrogen fumerate (Tiamutin,

Navartis, Bangladesh @ 30 gram in 100kg of feed daily for 5 days) and carbontetracycline (Chlorstechlin, Navartis, Bangladesh @ 300 gram in 100kg of feed daily for 5 days) were used as therapeutic measurement.
The dose of antibiotic, route of administration and the course of treatment were directed as per instructions. Electrolytes to correct ionic balance of the body fluids and acidification of gastrointestinal tract with acidifier (vinager@10ml per litre of water daily for 5 days) along with the course of antibiotics were also emphasized. Improving the sanitation in poultry houses and the management practices to avoid any furthermore stress were also suggested. No drug sensitivity test was done for the antibiotic selection. The response to treatment was noted.

RESULTS

Clinical findings/Cases history

Out of 13 incidences of NE, 9 in broiler, 5 in layer and 3 in cockerel flocks were detected during the course of study. The morbidity rate was about 100% and the mortality rate varied from flock to flock ranging from 4.58 – 11.82% which was detected from the farm records.

Table 2. Determination of average incidences and mortality rate of NE in poultry based on the types of birds

Types of birds	Age of birds ranging from	Rearing system	Total incidences	Total population of birds	Total birds died	Average mortality rate
Broiler	2 - 6 weeks	Litter	9	4156	294	7.07%
Layer	9 - 14 weeks	Litter	5	2858	131	4.58%
Cockerel	9 - 12 weeks	Litter	3	1811	214	11.82%

Biosecurity was usually poor, feeding was at libitum. Highest mortality rate was found in cockerel (11.82%) followed by broiler (7.07%) and layer (4.58%), respectively. The increased incidences of NE were recorded in the flocks previously exposed to enteric infection, mainly with coccidiosis.

Clinical signs

The major clinical signs observed during physical visit of the farms and also detected from the farmer's complaints. The affected birds showed severe depression, diarrhoea (shooting type), huddling, reluctance to move, ruffled feathers, and sudden death. Death recorded inspite of history of a good bodily condition and good appetite. The main complaints of the most farmers were the quick wet litter, shooting type diarrhoea and subsequently death.

Necropsy and histopathological findings

At necropsy, the birds generally showed good bodily conditions, but severely dehydrated. The skin was tightly attached with the body, tearing of underlying muscles during the postmortem skinning and darkened breast muscles were found.

The striking gross morbid lesions were located at mid-small intestine (jejunum and ileum) (Figure 2), where the enteric mucosa was abnormally thickened like yellow brownish diphtheritic membrane, varying degrees of haemorrhages, ballooning of the intestine, expulsion of foul smelling gas when opened. Large amount of necrotic enteroepithelial debris in the lumen including flecks of blood occasionally was also seen.
The lesion of intestines were histopathologically characterized as severe necrosis of enteroepithelial cells with marked desquamation, increased cellular infiltration in lamina propria, fibrin mixed with cellular debris adherent to intestinal mucosa.

BACTERIOLOGICAL FINDINGS

Large numbers of gram positive bacilli were found on Gram stained impression smears of intestinal lesions. Direct smear of faeces and lesions were done and coccidial oocysts were found in 2 cases but were not pathologically significant.

The clostridial organisms were isolated from the intestinal lesions by culturing and incubated anaerobically at 37ºC on blood agar media, where the organisms were readily grown and produced colonies characterized by the inner zone of haemolysis and the outer zone of partial haemolysis. Identification of the organisms was done allowing reactions in different biochemical media, where the organisms fermented carbohydrate and produced acid. They did not ferment mannitol.

THERAPEUTICAL FINDINGS

The affected flocks were therapeutically managed with oxytetracyclin, doxycycline and sulphonamide group of drug (Suphadiazine and trimethoprim combination).
Variable therapeutic responses of the different flocks to the drugs were found (Table 3), but better response to oxytetracycline treatment was not yet known.

DISCUSSION

Necrotic enteritis in commercial chickens (broiler, layer, and cockerel) was investigated based on clinical, pathological, microbiological and therapeutical findings. The disease was diagnosed on the basis of clinical

signs, necropsy findings, histopathological examinations, isolation and identification of organisms by bacteriological examinations, the positive therapeutic response of the affected flocks to commercial antibiotics.

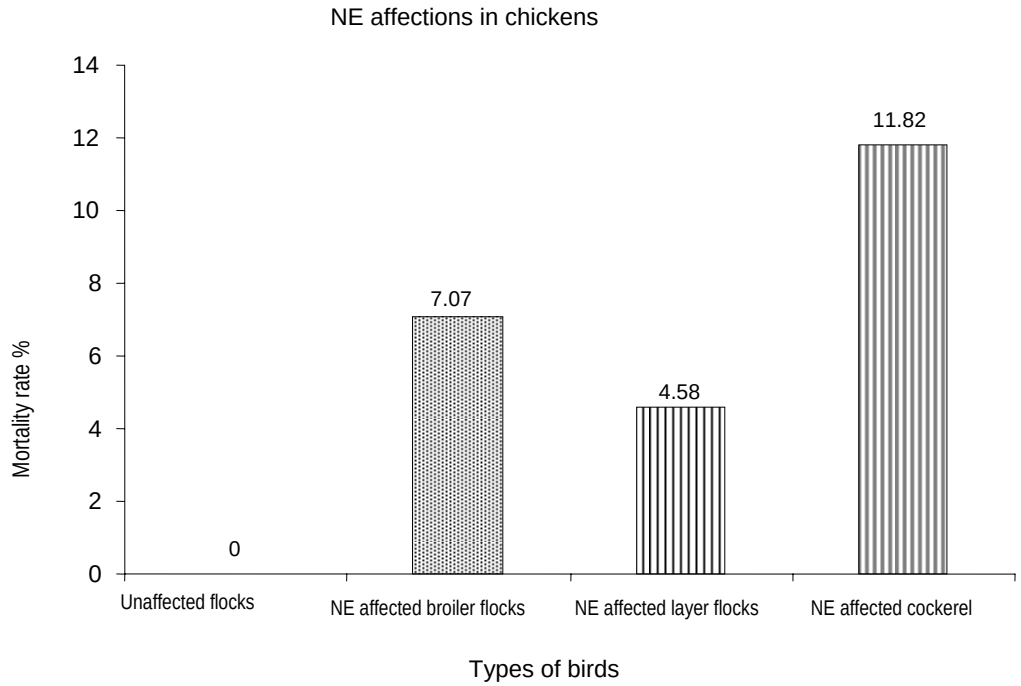

Figure 1. Graphical representation of average mortality rate of the different types of birds affected with NE including a group of unaffected flocks

Necrotic enteritis is an acute bacterial infection primarily of chickens. The disease was found endemically. The morbidity rate of the affected flocks was around 100%, but mortality was varied from farm to farm and the types of birds. NE was more commonly found in cockerel (11.82%) followed by broiler (7.07%) and layer (4.48%), respectively. The increased incidences of NE were recorded in the flocks previously exposed to coccidiosis and/or Gumboro disease.

Coccidial infection is a well documented predisposing factor for NE (Shane *et al.*, 1985; Frame and Bickford, 1986). Colonization of the small intestine by coccidia leads to the mucosal damage, which can provide a surface for CP to proliferate (Al-Sheikhly and Al-Saieg, 1980; Shane *et al.*, 1985). Lesions produced by *Eimeria brunetti* can be similar to those in necrotic enteritis, but uncomplicated coccidiosis is seldom as acute or severe (Fraser *et al.*, 1998). Coccidiosis can readily be confirmed by direct faeces or mucosal scrap examination under microscope, where insignificant numbers of coccidial organisms were identified. Barker and Van Dreumel (1993) stated that many factors may influence the severity of disease associated with enteric pathogens including virulence, host susceptibility, immune status, infective dose and diet.

The major clinical signs of the affected birds (diarrhoea, depression, huddling and sudden death) were more or less similar to those described by many authors (Calnek, 1997). The most farmer's complaints were the quick wetting of litters. Diarrhoea in such cases could result from a combination of fluid loss from localized inflammation and decreased fluid absorption due to disruption of the enteroepithelial barrier (Barker and Van Dreumel, 1993). Diarrhoea has been identified as a common clinical signs relating to CP infection among the poultry professionals (Carrier, 2000).

Dehydration due to diarrhoea and tearing of underlying muscles during the postmortem skinning due dehydration are well recognized pathogenesis (Radostits *et al.*, 2000). The duration of clinical signs in this study varied and its exact oetiology was not yet known. But sudden death commonly with no premonitory signs is noticed (Long, 1973; Tsai and Tung, 1981; Shane *et al.*, 1985; Ficken and Wages, 1997).

The intestine showed increased diameter (ballooning of the intestine) due to deposition of excess gas (Figure 2). The striking postmortem lesions were found in the mid-small intestine (jejunum and ileum), where the enteric mucosa was markedly thickened revealed yellow brownish diphtheritic membrane with haemorrhages occasionally (Charlton, 2000; Vegad and Katiyar, 2003).

The enterohistopathology in the present study was more or less similar described elsewhere (Charlton, 2000; Vegad and Katiyar, 2003). The specific location of lesions in intestine and absence of significant number of coccidia on direct microscopic examination of faeces and tissue smears clearly differentiating NE from coccidiosis.

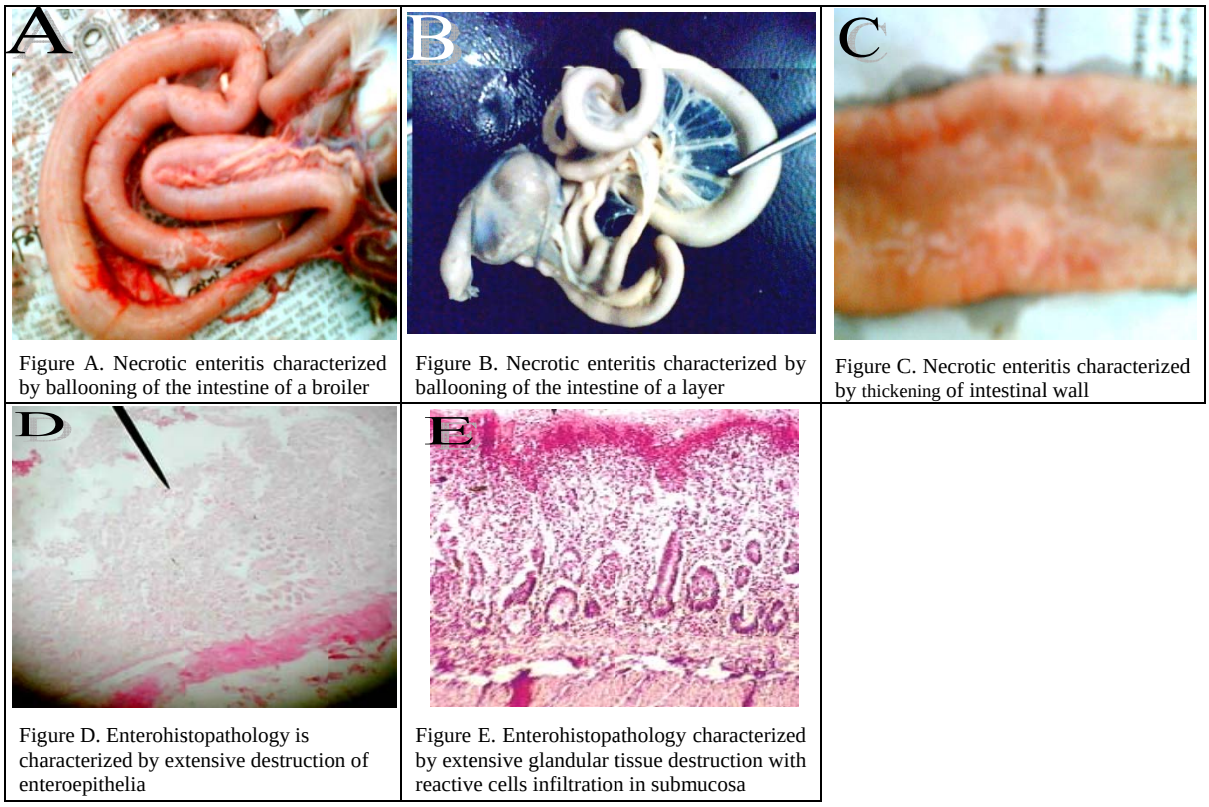

Figure A. Necrotic enteritis characterized by ballooning of the intestine of a broiler

Figure B. Necrotic enteritis characterized by ballooning of the intestine of a layer

Figure C. Necrotic enteritis characterized by thickening of intestinal wall

Figure D. Enterohistopathology is characterized by extensive destruction of enteroepithelia

Figure E. Enterohistopathology characterized by extensive glandular tissue destruction with reactive cells infiltration in submucosa

Figure 2: Different characteristic features of the intestine of NE affected birds and their causal agents

The gross lesion in usually confused with coccidiosis (Long et al, 1974; Porter, 1998) and confirmation was made on the gross lesions supported by histopathological and microbiological examination of tissues and faeces.

Table 3. Therapeutic findings of the different flocks treated with commercial drugs

Trial No.	Commercial preparation of drugs	Dose, route of administration and course of treatment	Therapeutic responses
1	Trimethoprim-sulphadiazine combination	1 ml per 5 litre of water daily orally for 5 days	+
2	Oxytetracyclin	3 gram per 5 litre of drinking water daily for 5 days	+++
3	Doxycycline	1gram per 2 litre of drinking water daily for 5 days	+
4	Tiamulin hydrogen fumerate 45%	35 gram in 100 kg of feed daily orally for 5 days	+++
	Carbon tetracycline 15%	300 gram in 100 kg of feed along with Tylosine phosphate daily orally for 5 days	
5	Trimethoprime, sulphadiazine and erythromycine combination	1 ml per litre of water daily orally for 5 days	++
6	Enrofloxacillin	1gram per litre of drinking water daily for 5 days	+

+++: Best response, ++: Better response, +: Good response

Table 4. Biochemical properties of the organism isolated from the intestinal lesions

Media	Biochemical properties of the organism
Dextrose	Fermentation with acid production
Mannose	Fermentation with acid production
Lactose	Fermentation with acid production
Sucrose	Fermentation with acid production
Mannitol	No reaction
Gelatin	Hydrolysis of gelatin
Milk	Digestion of milk
Indole production test	No reaction
Isolated organisms: Clostridia	

A presumptive diagnosis was made on the basis of examination of Gram stained impression smears of intestinal mucosa (Ficken and Wages, 1997). Clostridial organisms were isolated and identified by culturing in blood agar media and differentiated performing biochemical reactions in different media (Wilson *et al.*, 1996) (Table 4). Clostridium organisms are rod shaped, paired or shortly chained anaerobic organisms.

The affected flocks were tried to therapeutically manage to suggest the adnministration of oxytetracyclin, doxycycline, sulphadiazine-trimethoprim combination, tylosin along with carbontetracycline mainly based on necropsy findings and without any efficacy test. Better response to oxytetracycline and tylosin along with carbontetracycline was not yet known. Wilson *et al.*, (1996) reviewed that treatment with tylosin was effective in controlling outbreaks, but relapse occurred. Along with antibiotics, electrolytes and improving managements were emphasized to overcome the situations.

CONCLUSION

Investigation of NE in commercial chickens based on the findings as stated above with certainly help in proper diagnosis of the disease which causes considerable economic loss to the poultry farmers. So, this study will also alert poultry professionals about the disease, help to dictate specific medication as well as to adopt prevention and control strategies.

ACKNOWLEDGEMENTS

The authors are grateful and would like to thank Abu Hannan Md. Sadek (Soton), M/S Shachcho Enterprize, Chick dealer, Ranirbandor, Chairirbandor, Dinajpur and Sree Sukdev, Maa Tara Poultry Feeds, Sayedpur, Nilphamari district and the associated poultry farmers for their cooperation during conducting the research.

REFERENCES

Al-Sheikhly, F. and Al-Saieg, A. (1980). Role of coccidia in the occurrence of necrotic enteritis of chickens. *Avian Diseases*, 24 (2): 324-333.

Barker, I.K. and Van Dreumel, A.A. (1993). Intestine. Pages 1-318 In *Pathology of Domestic Animals* (4th edn.)K.V.F. Jubb, P.C.Kennedy and N. Palmer eds. Academic Press, Orlando FL.

Benno, Y., Endo, K. and Mitsuoka, T. (1988). Isolation of faecal *Clostridium perfringens* from broiler chickens and their susceptibility to eight antimicrobial agents for growth promotion. *Japanese Journal of Veterinary Science*, 50 (3): 832-834.

Benson, J.A. (1984). Manual of veterinary investigation laboratory techniques. Ministry of Agriculture, Fisheries and Food (3rd edn.). London. Volume 1, Reference Book 389.

Calnek, B.W. (edn.). (1997). *Diseases of Poultry*, 10th edn. Iowa State University Press, Ames, Ia.

Carrier, D. (2000). Clostridial enteritis global impact assessment and global MIC survey – a review. *Proceedings of the Poultry Enteritis Conference*. Elanco Animal Health. Montreal Que. 56 – 75.

Charlton, B.R. (2000). *Bacterial Diseases. In* Avian Disease Manual. 5th edn. The American Association of Avian Pathologists. U.S.A.

Dutto, G.A. and Devriese, L.A. (1980). Susceptibity of Clostridium perfringens of animal origin to fifteen antimicrobial agents. *Journal of Veterinary Pharmacology and Therapeutics*, 3: 227-236.

Ficken, M.D and Wages D.P. (1997). Necrotic enteritis. Pages 261-264 in Diseases of Poultry. 10th edn. B.W. Calnek, ed. Iowa State Press, Ames.

Frame, D.D. and Bickford, A.A. (1986). An outbreak of coccidiosis and necrotic enteritis in 16 week old caged reared layer replacement pullets. *Avian Diseases*, 30(3): 601-602.

Fraser, C.M., Bergeron, J.A. and Aiello, S.E. (1998).*The Merck Veterinary Manual.* A Handbook of Diagnosis, Therapy, and Disease Prevention and Control for the Veterinarian. (Eighth Edition). Merck & CO., Inc. press, U.S.A.

Hofshagen, M. and Kaldhusdal, M. (1992). Barley inclusion and avoparcin supplementation in broiler diets. 1. Effect on small intestinal bacteria flora and performance. *Poultry Science*, 71: 959-969.

Kaldhusdal, M. and Skjerve, E. (1996). Association between cereal contents in the diet and incidence of necrotic enteritis in broiler chickens in Norway. *Preventive Veterinary Medicine*, 28: 1-16.

Long, J.R. (1973). Necrotic enteritis in broiler chickens. I. A review of the literature and the prevalence of the disease in Ontario. *Canadian Journal of Comparative Medicine,* 37: 302 – 308.

Long, J.R., Pettit, J.R. and Barnum, D.A. (1974). Necrotic enteritis in broiler chickens. II. Pathology and proposed pathogenesis. *Canadian Journal of Comparative Medicine*, 38: 467 – 474.

Luna, L.G. (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Animals*, 3rd edn. McGraw-Hill Book Company. New York.

McDougald, L. R. (2003). Coccidiosis. In Diseases of Poultry. 11th edn. Y. M. Saif. Iowa State Press, Ames.

McLeod, W.G., Rowland, A.C. and Sewell, M.M.H. (1981). *Handbook of tropical Veterinary Laboratory Diagnosis.* Centre for Tropical Veterinary Medicine University of Edinburgh.

Niilo, L. (1980). Clostridium perfringens in animal disease: A review of current knowledge. *Canadian Veterinary Journal*, 21 (5): 141-147.

Porter, R.E. (1998). Bacterial enteritides of poultry. *Poultry Science*, 77: 1159 – 1165.

Radostits, O.M., Blood, D.C., Gay, C.C., and Hinchcliff, K.F. (2000). *Veterinary Medicine*: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th edn. WB Sanders. London.

Shane, S.M., Gyimah, J.E., Harington, K.S. and Snider, T.G. (1985). Etiology and pathogenesis of necrotic enteritis. *Veterinary Research Communications,* 9: 269-287.

Stutz, M.W., Johnson, S.L. and Judith, F.R. (1983). Effects of diet, bacitracin and body weight restriction on the intestine of broiler chicks. *Poultry Science,* 62: 1626-1632.

Tsai, S.S. and Tung, M.C. (1981). An outbreak of necrotic enteritis in broiler chickens. *Journal of the Chinese Society for Veterinary Science*, 7: 13-17.

Vegad, J.L. and Katiyar, A.K. (2003). *Bacterial Diseases. In* A Textbook of Veterinary Special Pathology (Infectious Diseases of Livestock and Poultry). International Book Distribution Co. U.P. India.