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

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

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 37°C 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 @ 1 gram 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 (Tiamulin,
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

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

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 enteropithelial debris in the lumen including flecks of blood occasionally was also seen. The lesion of intestines were histopathologically characterized as severe necrosis of enteropithelial 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 37°C 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.

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 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-Saeeg, 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 litter. 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 sign relating to CP infection among the poultry professionals (Carrier, 2000).

Dehydration due to diarrhoea and tearing of underlying muscles during the postmortem skinning due to dehydration are well recognized pathogenesis (Radostits et al., 2000). The duration of clinical signs in this study varied and its exact otiology 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).

![Figure 1. Graphical representation of average mortality rate of the different types of birds affected with NE including a group of unaffected flocks](image-url)
The enterohistopathology in the present study was more or less similar described elsewhere (Charlton, 2000; Vegad and Katyar, 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](image1)

![Figure B. Necrotic enteritis characterized by ballooning of the intestine of a layer](image2)

![Figure C. Necrotic enteritis characterized by thickening of intestinal wall](image3)

![Figure D. Enterohistopathology is characterized by extensive destruction of enteroepithelia](image4)

![Figure E. Enterohistopathology characterized by extensive glandular tissue destruction with reactive cells infiltration in submucosa](image5)

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

The gross lesion is 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

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Commercial preparation of drugs</th>
<th>Dose, route of administration and course of treatment</th>
<th>Therapeutic responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trimethoprim-sulphadiazine combination</td>
<td>1 ml per 5 litre of water daily orally for 5 days</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Oxytetracyclin</td>
<td>3 gram per 5 litre of drinking water daily for 5 days</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Doxycycline</td>
<td>1 gram per 2 litre of drinking water daily for 5 days</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tiamulin hydrogen fumarate 45%</td>
<td>35 gram in 100 kg of feed daily orally for 5 days</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Carbon tetracycline 15%</td>
<td>300 gram in 100 kg of feed along with Tylosine phosphate daily orally for 5 days</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>Trimethoprim, sulphadiazine and erythromycin combination</td>
<td>1 ml per litre of water daily orally for 5 days</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Enrofloxacin</td>
<td>1 gram per litre of drinking water daily for 5 days</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ : Best response, ++ : Better response, + : Good response
Table 4. Biochemical properties of the organism isolated from the intestinal lesions

<table>
<thead>
<tr>
<th>Media</th>
<th>Biochemical properties of the organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>Fermentation with acid production</td>
</tr>
<tr>
<td>Mannose</td>
<td>Fermentation with acid production</td>
</tr>
<tr>
<td>Lactose</td>
<td>Fermentation with acid production</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Fermentation with acid production</td>
</tr>
<tr>
<td>Mannitol</td>
<td>No reaction</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Hydrolysis of gelatin</td>
</tr>
<tr>
<td>Milk</td>
<td>Digestion of milk</td>
</tr>
<tr>
<td>Indole production test</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

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 administration 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.

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Necrotic enteritis in chickens: pathological, bacteriological and therapeutical investigation


