PREVENTION OF NECROTIC ENTERITIS USING C. PERFRINGENS VACCINE IN MALE LAYER CHICKENS.

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SUMMARY

C. perfringens vaccine was used for prevention of necrotic enteritis (NE) in male layer chickens in presence of predisposing factor. Vaccinated chicken groups subjected to predisposing factor showed improved results as compared with non vaccinated group. Three days average body weights of treated infected showing that birds of coccidia vaccine having the highest values from the 1st -7th dpi, followed by coccidia vaccine group with aflatoxin group, while infected non vaccinated group showed the lowest values.

Regarding weekly and cumulative body weight gain (BWG), feed intake (FI) and feed conversion rate (FCR) of chickens groups given NE vaccine and challenged with C. perfringens culture. Vaccinated groups were higher than infected control groups. Bursa and thymus lesions in vaccinated groups were milder than non vaccinated. C. perfringens vaccine resulted in lowering lesion score in both intestine and liver scores till the 9th dpi as compared with nonvaccinated group. Pathological finding there was a good correlation comparing between both gross and macroscopically finding especially in intestinal changes.

The role of NE vaccine in prevention of NE disease needs more investigations especially in presence of immunosuppressive factors. Single dose of NE vaccine had role in minimizing the effect of challenge with C. perfringens broth culture on performance, gross and histopathological lesions.

The used NE vaccine had a role in prevention of NE in presence of aflatoxins and/or coccidia vaccines. This findings required more investigations especially in presence of immunosuppressive factors.

INTRODUCTION

Outbreaks of NE can be prevented or treated by the use of in-feed antibiotics, but their use is now being questioned in many countries. However, the current debate regarding the prophylactic use of antibiotics in animal diets necessitates a better understanding of factors that influence intestinal colonization by C. perfringens as well as the pathophysiological consequences of its growth (Collier et al., 2003; Williams, 2005). Also coccidiostat had been recommended as a toile for

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disease control (Jackson et al, 2003; Johansson et al., 2004; Williams, 2005).

Withdrawal of antimicrobial growth promoters and ionophore coccidiostats has been accompanied by resurgence in incidence of NE. Therefore, the production and use of NE vaccine had great attention in the last years as it was reported that Active and passive immunity through vaccination against C. *perfringens* and its toxins appears to offer good protection against infection. Immunization of chickens with a virulent strain of C. *perfringens* followed by an antibiotic treatment protected birds against a challenge infection with C. *perfringens* (Saif et al., 2003 and Thompson et al., 2006).

Immunization of broiler breeders with alpha-toxin vaccines produces an antibody response that appears to be protective in progeny against subclinical C. *perfringens*-associated NE and hepatitis Lovland et al. (2004).

Knowledge and necessary further experiments are identified. Insights are provided regarding the prevalence in commercial flocks and interactions between coccidiosis, aflatoxicosis and NE. This study was carried out to use C. *perfringens* gel vaccine to protect chickens against experimental infection with Toxogenic C. *perfringens* isolate in the presence or absence of aflatoxins and/or coccidial vaccines.

**MATERIAL and METHODS**

**Chicks:**
One handed and eighty, one day old male chicks were obtained from El Wadi group, Giza, Cairo was used in this work.

**Ration:**
Commercial balanced ration produced by El-Ahram poultry Company was used for the experimental chickens. The used ration was free from feed additives and mycotoxins.

**Vaccines:**
1. **Newcastle (ND), infectious disease (IB) and Infectious bursal disease (IBD) vaccines:**
   Live preventive vaccines were used via eye drop instillation in all used chicken groups. Combined ND and IB live commercial vaccine (**MA 5+ clone 30**), Holland list No 08834fj01 and IBD intermediate live commercial vaccine (**Gumboro D78**) list No 008828dj01. These vaccines were produced by Intervet international B.V, Boxmeer, Holland.
2. **Coccidiosis vaccine:**
   Live commercial coccidiosis vaccine (**Coccivac-D**) produced by Schering plough animal, health, and Millsboro, Delaware, USA lot No 167/08.
3. **C. perfringens Vaccine:**

Chicken NE gel vaccine was obtained from Vet, Serum and Vaccine Research Inst., Abassia, Cairo. The producer instruction for vaccine use stated that the 1<sup>st</sup> dose 0.5 ml at 2 weeks an 2<sup>nd</sup> 1ml at 2 month of age via s.c injection.

**Clostridial culture suspension:**

A 48 hours culture in the cooked meat medium was prepared from C. perfringens identified pathogenic isolate by **Hamouda et al. (2010)**. The culture suspension were centrifuged for one hour at 4000 rpm, gram stained smear made from sediment were examined microscopically to ensure purity. The sediment was washed three times in saline , and then resuspended in thioglycollate medium. the plates count technique **Cruikshank et al.,(1975)** was used for determination of the viable count of cells per ml of suspension.

**Gross pathological lesions:**

Gross lesions in sacrificed chicken were given scores according to **AL-sheikly and Truscott (1976)** as follows: - : Grossly normal organ. +: Mild infection. ++: Congested. +++: Necrotic lesions.

**Histopathological examination:**

Tissue samples were taken at 3, 6, 9, 12 and 15 days post infection (dpi) from infected and control chicken groups immediately after cervical dislocation. Organ specimens were obtained from the different parts of the small intestine 1 - 5 cm long, pieces about 0.5 – 2 gm from liver, thymus and bursa. All the specimens were fixed in 10% Formol saline at room temperature for at least 2 days before processing. The tissues including the intestine were trimmed and then embedded in paraffin blocks for sectioning. Section 5 µm thick were routinely stained with hematoxyline and eosin and examined microscopy for histopathological lesions as compared with non treated controls.

**Aflatoxin:**

Contaminated corn with 20 mg / 1 kg aflatoxine was kindly supplied by **Prof. Dr M. M. Amer**; professor of poultry disease, faculty of veterinary medicine, Cairo University. The contaminated corn was added to chicken ration in dose of 5g/kg of used ration.

**Experimental design:**

The used 180, 1- day old chicks were floor reared and fed commercial balanced ration without feed additives. At the 4<sup>th</sup> day of age the chicks were randomly divided into 6 equal groups; 30 chicks each. Each group was reared in clean separated room and given feed and water adlibitum. All groups were given ND with IB and IBD at the 5<sup>th</sup> and 9<sup>th</sup> day of age via eye drop; respectively.
At the 4th day chicks of groups 2 and 4 were given coccida vaccine by eye drop instillation of 0.05 ml/chicks containing 10 immunizing dose. Chickens of groups 3 and 4 were given aflatoxin at the dose of 5µg/kg in ration from the 24th day of age to the end of experiment.

At the 15th days of age birds of groups 2-5 were given NE vaccine by s.c injection with dose of 0.5 ml/chicks.

At age of 31 days birds of groups 2-6 were orally inoculated each with 3ml/chicks of broth whole C. perfringens culture containing (3x10⁹ CFU/ml), while birds of group 1 were left as nontreated negative control group.

All groups were subjected to daily observation for clinical signs and/or mortalities with recording of average BW From the 1st day and every 3 days till the 15th dpi. Average weekly BWG and FI for calculation of FCR were recorded during the 15 dpi. One bird/group was randomly sacrificed at 3, 6, 9 and 12 days as well as 10 birds at the 15th dpi for post-mortem with recording of lesions and collection of tissues for histopathological examination. The obtained results are shown in tables (1 and 2), figs (1and 2) and plates (15-20).

RESULTS

There were no marked clinical signs or mortality could be detected during this experiment. Average BW of all chickens groups was nearly the same without marked difference between treated vaccinated groups and the control negative and positive at the 1st dpi (Table1, Fig1), at the 4th and 7th dpi the average BW of treated groups given NE vaccine showed that the group 4 and 5 were the highest values; while at the 11th dpi and 15th dpi all groups were moderately similar. The average BW of infected no vaccinated group 6 was the lowest value at 1st, 4th, 7th and 15th dpi compared to all groups followed by that of the control negative group.

The BWG of infected groups given NE vaccine were higher than the infected non vaccinated group and the control negative group in the 1st week with highest value of group 3 and 4 followed by that of the group 2 and 5 (Table 2); while in the 2nd week the group 3 and 2 followed by the group 5 and 4 were higher than the infected no vaccinated group and the all groups were lower than control positive group. in the other hand the total BWG of the infected no vaccinated group was the lowest value compared to all groups followed by the control negative group.

Regarding the FCR of infected groups given NE vaccine, the group 3 and 4 were the lowest value followed by that of the group 2 and 5, and the all were lower than infected no vaccinated group in the 1st week (Table2, Fig 2). In the 2nd week the group 4 was the highest value; while the group 3 followed by the group 5 and 2 were lower than the infected non vaccinated group. In the other hand the control negative group was the lowest value compared to all groups. The total FCR the infected vaccinated groups were
lower than the infected no vaccinated group with lowest value of group 3 followed by that of the group 5, 2 and 3. The control negative group was higher than group 3 and lower than the other remaining groups.

Control negative birds (group 1) showed no detectable lesions. Chickens of group 2 given coccidia vaccine and NE vaccine showed haemorrhages in intestinal wall at 9 dpi and liver necrosis (plate 1 A and A1). Also, massive necrosis and haemorrhages in intestinal mucosa was seen at 12th dpi (plate 1C). Examined chickens at the 15th dpi having haemorrhages in intestinal wall, liver necrosis and cecal core (plate 1 C, C1 and C2). Group 3 showed distended hemorrhagic cecum at 9th dpi (plate 2 A) as well as; massive mucosal necrosis and haemorrhages, liver necrosis and distended cecum at 12th dpi (plate 2 B). Group 6 (the control infected non vaccinated) showed haemorrhages in intestinal wall at 3rd dpi, massive hemorrhage and necrotic foci in intestinal mucosa at 6th dpi; liver necrosis with hemorrhages in intestinal wall was detected at 9th and 12th dpi; necrosis in intestinal wall was additionally seen at 12th dpi. Intestinal lesions at 15th dpi were very mild.

Table (1): Average body weight of groups infected with NE vaccine after different treatment.

<table>
<thead>
<tr>
<th>Z</th>
<th>Treatment</th>
<th>Weight post infection</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 day</td>
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<td></td>
<td></td>
<td>4 days</td>
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<td>7 days</td>
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<td>11 days</td>
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<td>15 days</td>
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<td>----</td>
<td>-----------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Control negative</td>
<td>331.4 ± 28.4</td>
</tr>
<tr>
<td>2</td>
<td>Co. vaccine + C. p Vaccine + C. p culture</td>
<td>336.9 ± 22.3</td>
</tr>
<tr>
<td>3</td>
<td>Aflatoxin + C. p Vaccine + C. p culture</td>
<td>331.7 ± 37.9</td>
</tr>
<tr>
<td>4</td>
<td>Co. vaccine + Aflatox + C. p Vac + C. p culture</td>
<td>330.7 ± 28.5</td>
</tr>
<tr>
<td>5</td>
<td>C. Vaccine + Cp. culture</td>
<td>332.9 ± 47.8</td>
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<tr>
<td>6</td>
<td>C. p culture (infected control)</td>
<td>324.8 ± 34.1</td>
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Table 1: Average body weight of groups infected with NE vaccine after different treatment.
Table (2): Weekly and total body weight gain (BWG), feed intake (FI) and feed conversion rate (CR) of chicken groups given NE vaccine (NEv) and challenged with C. perfringens culture.

<table>
<thead>
<tr>
<th>NO</th>
<th>Treatment</th>
<th>Weekly average</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
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<tr>
<td></td>
<td></td>
<td>BWG</td>
<td>FI</td>
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<tr>
<td>1</td>
<td>Control negative</td>
<td>82.5</td>
<td>191.37</td>
</tr>
<tr>
<td>2</td>
<td>Coccida vacc.+NE v</td>
<td>100.9</td>
<td>234.48</td>
</tr>
<tr>
<td>3</td>
<td>Afla+NEv</td>
<td>106.5</td>
<td>213.33</td>
</tr>
<tr>
<td>4</td>
<td>Coccida vacc.+ afla+NEv</td>
<td>106.5</td>
<td>213.29</td>
</tr>
<tr>
<td>5</td>
<td>Vaccine</td>
<td>97.4</td>
<td>235.71</td>
</tr>
<tr>
<td>6</td>
<td>C. perfringens culture</td>
<td>85.9</td>
<td>227.58</td>
</tr>
</tbody>
</table>

Plate (1): Liver and intestinal lesion in sacrificed chickens given both Coccida and NE vaccines and infected with C. perfringens showing:


A1  B  C1

C2
Plate (2): Liver and intestinal lesion in sacrificed chickens given both aflatoxin and NE vaccine and infected with C. perfringens showing:

A: 9dpi; distended hemorrhagic cecum.  B: 12dpi; Massive mucosal necrosis and haemorrhages, liver necrosis and distended cecum

Gross lesion score in C. perfringens groups showing that the usage of C. perfringens vaccine resulted in lowering score in both intestine and liver scores till the 9\textsuperscript{th} dpi. Intestinal lesion score in group 6 increased to 3+ at 12\textsuperscript{th} and 15\textsuperscript{th} dpi.

Histopathological examination of tissue of group 2 (Cocc V+ cl culture+NE vac) showed no lesions at all intervals. Birds of group 3 showed focal necrotic area with leucocytic infiltration in liver at the 12-15 dpi (plate 3, 2). Intestine showed mucosal inflammation, submucosal fibrosis and congestion in 12-15 dpi (plate 4, 2). Bursa showed follicular haemorrhage (plate 5, 2) ; while thymus showed medullary congestion and haemorrhage in samples of 9-15 dpi. Results of group 4 were showed portal leucocytic infiltration at 12\textsuperscript{th} dpi (plate 3, 3) with focal necrotic area with mononuclear cells infiltration at 15\textsuperscript{th} dpi(plate 3, 3a), intestine haemorrhagic mucosa at 12-15 dpi (plate 4, 3), bursa showed interfollicular fibrosis at 12-15 dpi (plate5, 3), while congestion and haemorrhage was in thymus at 12-15 dpi (plate 6 , 3).

Group 5 liver showed healthy hepatic tissue at all intervals (plate 3, 4) .in the other hand the intestine showed submucosal oedema and congestion at 3-15 dpi (plate18,phot4). Bursa showed follicular haemorrhage at 3-15 dpi (plate 5, 4) .as well as, thymus (plate 6, 4) showed diffuse haemorrhage at 3-15 dpi.

Liver Sections of group 6 were showed dissociation and disorganization of hepatic parenchyma at 3\textsuperscript{rd} dpi (plate 3, 5) ; while the focal area of mononuclear cells infiltration was seen at 6-15 dpi (plate 3. 5a). At 3-15 dpi (plate 3, 5) intestine showed mucosal necrosis and leucocytic infiltration. Bursa showed necrotic follicles with interfollicular oedema at 3 dpi (plate 4, 5) with follicular disintegration at 9-15 dpi (plate 4, 5a).Thymus showed medullary necrosis in samples of 6-15 dpi (plate 4, 5a).
Pathological finding there was a good correlation comparing between both gross and microscopical finding especially in intestinal changes. Moreover the histopathological examination resulted in early detection of liver lesions.

Plate (3): Liver tissue sections stained with H&E in sacrificed chickens given NE vaccines and infected with C. perfringens showing:

1: 3-15 dpi; gr 2 apparently normal hepatic parenchyma (X200).
2: 9-15 dpi; Gr3 focal necrotic area with leucocytic infiltration (arrows) (X 200).
3: 12 dpi; Gr 4 portal leucocytic infiltration (L) (X200).
3a: 15 dpi; Gr 4 focal necrotic area with mononuclear cells infiltration (n)(X 400).
4: 3-15 dpi; Gr 5 apparently healthy hepatic tissues (X 400).
5: 3 dpi: Gr 6 dissociation and disorganization of hepatic parenchyma (arrows) (X 400).
5a: 6-15 dpi: Gr 6 focal area of mononuclear cells infiltration (m) (X×200).
Plate (4): Intestinal tissue sections stained with H&E in sacrificed chickens given NE vaccines and infected with C. perfringens showing:

1: 3-15 dpi; Gr 2 apparently normal histology of intestinal tissue (X 200).
2: 12-15 dpi; Gr 3 mucosal inflammation (arrows) and submucosal fibrosis (F) and congestion (C) (X 200).
3: 12-15 dpi; Gr 4 hemorrhagic mucosa (arrows) (X 200).
4: 3-15 dpi; Gr 5 submucosal oedema (e) and congestion (c) (X 400).
5: 3-15 dpi; Gr 6 mucosal necrosis (n) and leucocytic infiltration (L) (X 200).

Plate (5): Bursal tissue sections stained with H&E in sacrificed chickens given NE vaccines and infected with C. perfringens showing:

1: 3-15 dpi; Gr 2 apparently normal follicles (X 200).
2: 9-15 dpi; Gr 3 follicular haemorrhage (h) (X 200).
3: 12-15 dpi; Gr 4 interfollicular fibrosis (f) (X 200).
4: 3-15 dpi; Gr 5 follicular haemorrhage (arrows) (X 400).
5: 3 dpi; Gr 6 necrotic follicles (n) and interfollicular oedema (e) (X 200).
5a: 9-15 dpi; Gr 6 follicular disintegration (arrows) (X 400).
Plate (6): Thymus tissue sections stained with H&E in sacrificed chickens given NE vaccines and infected with C. perfringens showing:

1: 3-15 dpi; Gr 2 apparently healthy cortex and medulla (X 200).  
2: 9-15 dpi; Gr 3 medullary congestion (c) and haemorrhage (arrows) (X 200).  
3: 12-15 dpi; Gr 4 congestion (c) and haemorrhage (arrows)(X 200).  
4: 3-15 dpi; Gr 5 diffuse haemorrhage (h) (X 400).  
5: 6-15 days Gr 6 medullary necrosis (n) (X 100).  
5a: 6-15 days; Gr 6 higher magnification of the medullary necrosis (n) (X 400).

**DISCUSSION**

The incidence of C. perfringens associated NE in poultry has increased in countries that stopped using antibiotic growth promoters (Van immerseel, 2004), for that the vaccination could be a helpful tool in preventing NE in poultry. It is known that flocks with high titers of maternal antibodies against alpha toxin had lower mortality during the production period than flocks with low titers (Heier et al., 2001). The present study was carried out to investigate the role of NE vaccine in prevention of NE in presence of predisposing factors.

The both average body weight and average weekly body weight gain (table 1-1, fig1); of infected no vaccinated group 6 was the lowest value at compared to all groups followed by the control negative group. This result clearly showing the significance of vaccine on body weight. In addition the total feed conversion rate (table 2.fig2) of the infected vaccinated groups were lower than the infected no vaccinated group with lowest value of group 3 followed by that of the group 5, 2 and 4. The control negative group was higher than group 3 and lower than the other remaining groups. The results proved that NE vaccine prevents the adverse effect of infection, these results agreed with El-Meneisy et al., (2007) who study the preventive effect of locally prepared NE vaccine (Egypt) and demonstrated that vaccination of chickens with NE vaccine was associated with several
beneficial effects, as increased body weight gain, decreased feed conversion ratio and decreased in the number of underweight birds.

Gross lesion score in NE vaccinated groups (table 13) showing that the usage of NE vaccine resulted in lowering score in both intestine and liver scores till the 9th dpi. Intestinal lesion score in group 6 increased to 3+ at 12th and 15th dpi. This result agreed with Cooper et al., (2009) who reported that non vaccinated chickens had lesions score averaging 2.37, while average score in vaccinated chickens were 1.35. Also Kulkarni et al (2007) demonstrated that immunized groups against NE had significantly fewer chickens with lesions than the unimmunized control groups. In addition El-Meneisy et al., (2007) reported that intestinal lesions were higher in unvaccinated birds than in those receiving vaccine.

General histopathological examination of intestinal and liver tissue were mild in vaccinated groups; regardless the previous treatment; as compared with group 6. Also the detected lesions appeared mostly at later ages post infection in vaccinated groups (2-5).

Generally, Bursa showed follicular haemorrhage (plate 19, photo 2); while thymus showed medullary congestion and haemorrhage in samples of 9-15 dpi in all groups except group 6 where no vaccine the lesions appeared early.

Pathological finding there was a good correlation comparing between both gross and microscopical finding especially in intestinal changes. Moreover the histopathological examination resulted in early detection of liver lesions.

Single dose of NE vaccine had role in minimizing the effect of challenge with C. perfringens broth culture on performance, gross and histopathological lesions.

The used NE vaccine had a role in prevention of NE in presence of aflatoxins and/or coccidia vaccines. This findings required more investigations especially in presence of immunosuppressive factors.

**REFERENCES**


الملخص العربي:

استخدام لقاح ميت لالتهاب المعدة المتكرز في الوقاية من الالتهاب الضارة في وجود العوامل المهيّنة للإصابة. أظهرت المجموعات التي أعطيت اللقاح نتائج أفضل من المجموعة الضابطة، حيث الاختلافات في انخفاض الوزن ومعدلات التحويل الغذائي وكذا الآفات المرضية التشريحية والمجهرية.

كان لاعطاء اللقاح اثارة إيجابية واضحة في السبع أيام الأولى من العدوى في المجموعة التي أعطيت لقاح الكوكسيديا كعامل مهيّنة للمرض نسبيًا إلى المجموعات الأخرى. بينما كانت نتائج المجموعة الضابطة غير المحصنة أقل.

أدى أعطاء اللقاح لالتهاب المعدي المتكرز إلى تأخر ظهور الأعراض التشريحية والنتيجة إلى مابعد اليوم السابع، كما لوحظ اثار الإيجابي الواضح للفائض على الآفات التشريحيّة المرضية لغبني البرسا والثامث. امكن التعرف على اثار إيجابية لاستخدام اللقاح المثبّت لالتهاب المعدي المتكرز بالمجهر. استخدام اللقاح يحتاج إلى المزيد من الدراسات خاصة في وجود العوامل المهيّنة لتحقيق الاستخدام الأمثل له.