Prevention of Necrotic Enteritis Using C. Perfringens Vaccine in Male Layer Chickens

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Received: 10/04/2011 Accepted: 11/05/2011

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 1 th dpi as compared with nonvaccinated group. Pathological finding showed that there was a good correlation comparing between both gross and macroscopical 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 finding requires

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 and Williams, 2005). Also coccidiostat had been recommended as tole for

Key words: Necrotic enteritis- chickens-C. perfringens- Mycotoxin, coccidian vaccine, Necrotic enteritis vaccine.

disease control (Jackson et al, 2003; Johansson et al., 2004 and 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 few 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 toxigenic C. perfringens isolate in the presence or absence of aflatoxins and/or coccidial vaccines.

MATERIALS AND METHODS

Chicks:

One handed and eighty, one day old male chicks were obtained from El Wadi group, Giza, 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:

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 08834fj01and 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 1st dose 0.5 ml at 2 weeks an 2nd 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 g 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 examined microscopically and 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, 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 4th 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 vaccines at the 5th and 9th 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 administered 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 3), Figs (1 and 2) and Plates (1-6).

RESULTS AND DISUSSIOIN

incidence perfringens of C. The associated NE in poultry has increased in countries that stopped using antibiotic growth promoters (Van immerseel, 2004) therefore; vaccination could be a helpful tool in preventing NE in poultry. It is know 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 use chickens were aged 31 days and received the vaccine at the age of 2 weeks. Natural outbreaks of NE have

been reported in 2-7 weeks old chickens (Bains, 1968 and Cygan and Nawak, 1974).

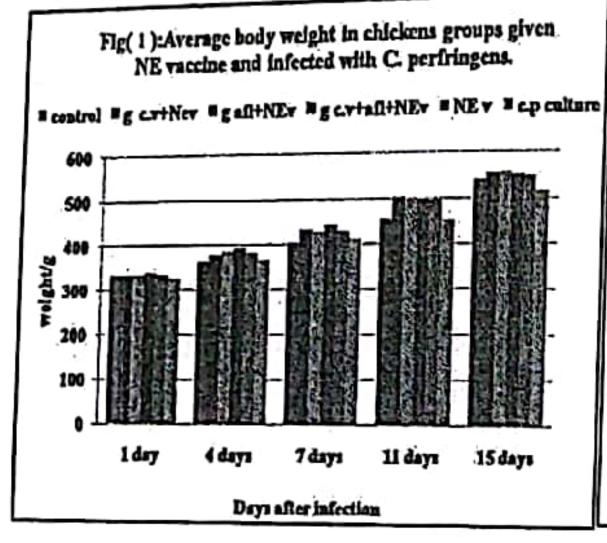
There were no marked clinical signs or mortality could be detected in both vaccinated challenged, positive and negative control groups. This result can be attributed to the infective dose was not able to induce clinical disease or to the nature of *C. pereferanges* as previously reported by Pedersen *et al.* (2003), Olkowanski *et al.* (2006) and Amer *et al.* (2010) who failed to induce mortality or apparent clinical signs of NE despite inoculation of a high doses of C. *perfringens*. Furthermore, Pedersen *et al.* (2003) and

Olkowanski et al. (2006) failed to induce mortality or clinical signs of NE despite inoculation of very high doses of C. perfringens and in short term exposure using a single and high dose. Pedersen et al. (2008) and Amer et al. (2010) who used coccidia vaccine and or aflatoxins at 10 times the recommended dose to establish infection with C. perfringens no mortality was detected and chickens developed the subclinical form of NE.

Three days recorded average body weight gain (ABWG) of all chickens groups was nearly the same without marked difference between treated vaccinated groups and

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

ດ		Weight post infection						
25 6	Treatments	1 day	1 day 4 days 7 days		11 days	15 days		
No.		$M \pm SD$	$M \pm SD$	$M \pm SD$	$M \pm SD$	$M \pm SD$		
1	Control negative	331.4	362.9	403.9 31.5	453.7	539.2 38.8		
	Control negative	28.4	31.6	A450 - 553*	32.5			
2	Co. vaccine + C. p Vaccine	336.9	393.5	443.3 27.8	497.7	549.2 42.6		
-	+ C. p culture	22.3	25.9	11313 2710	51.5	* ***		
3 Aff	Aflatoxin + C. p Vaccine +	331.7	376.6	432.6 55.7	501.2	552.8 44.6		
	C.p culture	37.9	43.1	452.0 55.7	60.4			
. (Co. vaccine + Aflato + C. p	330.7	384.8	427.2 35.7	500.4	555.9 42.2		
4	Vac + C. p culture	28.5	31.8	727,2 30.7	44.2			
5		332.9	381.8	429.3 54.3	496.9	546.8 69.9		
	C. Vaccine + Cp. culture	47.8	53.4	427,5 5,115	65.6	7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7		
7	C. p culture	324.8	364.8	410.7 42.0	452.9	514.4 60.4		
6	(infected control)	34.1	36.9	410.7 42.0	47.8			



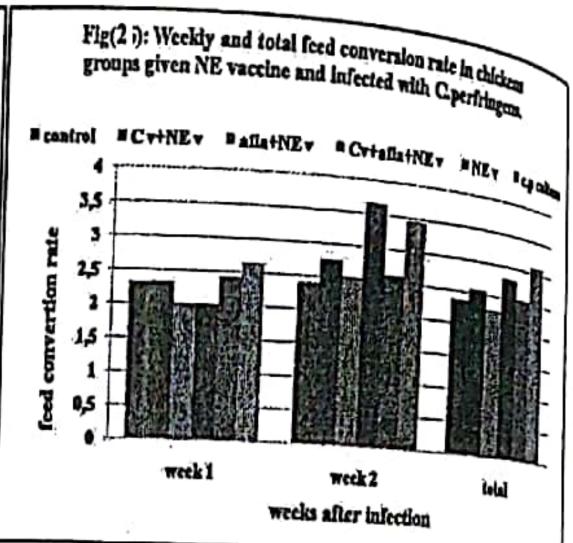


Table (2): Weekly and total body weight gain (BWG), feed intake (FI) and feed conversion rate (CR) of chicken groups given NE vaccine (NEv) and challenged with C. perfringens culture.

ଦ୍ର	Treatments		Weekly average							
Gr. NO		Week 1			Week 2			Total		
_		BWG	FI	CR	BWG	FI	CR	BWG	FI	CR
1	Control negative	82.5	191.37	2.31	135.5	325.92	2.4	2180	517.29	2.3
2	Coccida vacc.+NE v	100.9	234.48	2.32	121.2	334.61	2.76	222.1	569.09	2.5
3	Afla+NEv	106.5	213.33	2.00	128.7	325.00	2.52	235.2	538.33	2.28
4	Coccida vacc.+ afla +NEv	106.5	213.29	2.00	103.9	380.00	3.65	210.4	592.29	2.81
5	Vacine	97.4	235.71	2.42	117.5	307.69	2.61	214.9	543.4	2.52
	C. perfringens culture	85.9	227.58	2.64	103.7	359.25	3.46	189.6	586.83	3.09

controls at the 1st dpi (Table1, Fig1). Coccidia vaccine groups 2 showed the highest AVBG 393.5 ± 25.9g and 443.3 ± 27.8g at 4 and 7 dpi. The result can be attributed to the clostridia vaccine. As Pedersen *et al.* (2008)

and Amer et al. (2010) who reported that chickens received both coccidia vaccine and C. perfringens developed the subclinical form of NE as demonstrated by low growth rate. Aflatoxin group 4 (500.4 ± 44.2 and 555.9 ±

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42.2g) and group 3 (501.2 ± 60.4 and 552.8 ± 44.6g) showed higher ABWG at 11 and 15 dpi. ABWG of infected no vaccinated group 6 was the lowest value at 1st, 4th, 7th and 15th dpi compared with all groups followed by the control negative group. This result may explain the stimulating effect of small dose of aflatoixin as reported by Calabrase (2002), Diaz et al. (2008) and Amer et al. (2010) who reported that low levels of aflatoxin in feed consumed may act as growth stimulant to chickens.

Weekly calculated FCR (Table 2 fig 2) in the 1st week of grs 3 and 4 were the best (2.0) followed by that of the group 1 (2.31), group 2 (2.32) and all were better than gr 6 (2.61). In the 2^{sd} week group 4 was the lowest (3.65) followed by group 6 (3.46) while the best was the control group 1 (2.4). The results of coccidian vaccine groups 2 and 4 indicate the role of high vaccine dose in reduction of FCR. While the improved results in the 1st week can be attributed to *C. perferingens* vaccine.

Total FCR of Aflatoxin group 3 was (2. 28) higher than control negative group 1 (2.37) and C. perferingens vaccine group 5 (2.52) (table 2 fig 2); while the control positive group was the best (2.37). Infected group 6 showed the lowest FCR (3.09). This result can support the stimulating effect of Aflatoxin (Calabrase, 2002; Diaz et al., 2008) and Amer et al., 2010). Furthermore, the lower result of group 6

indiocate the inuction of subclinical infection (Kaldhusdal et al., 2001; Hofacre et al., 2003 and Amer et al., 2010). The results of coccidia and aflatoxin groups proved that NE vaccine prevents the adverse effect of infection, these results agreed with El-Meneisy et al., (2007) who studied 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.

Sacrificed 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 1, C). Examined chickens at the15th dpi having haemorrhages in intestinal wall, liver necrosis and cecal core (Plate 1, C; C1 and C2). Simillar lesions were detected in using coccidia vaccine in induction of NE (Williams, 2002; Pedersen et al., 2008 and Amer et al, 2010). Birds of 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). Similar findings were reported by Carnaghan et al. (1966), Park et al. (1994) and

Olkowski et al. (2006). 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 also seen at 12th dpi. Intestinal lesions at 15th dpi were very mild. These lesions were also reported in subclinical cases of NE infections by AI-Sheikhly and Truscott (1977), Tsai and Thug (1981) Lovland and Kaldhusdal (2001). The detected mild lesions in vaccionated groups can be induced under the control of immunity against C. perfringens.

Gross lesion score (Table 3) in C. perfringens challenged groups showed that the usage of C. perfringens vaccine resulted in

lowering score in both intestine and liver scores till the 9 th dpi. This fiunding support the value of vaccine in presence of stresses as reported by El-Meneisy et al., (2007) reported that intestinal lesions were higher in unvaccinated birds than in those receiving vaccine. Intestinal lesion score in group 6 increased to 3+ at 12th and 15th dpi as a result of infection (Al-Sheikhly and Al-Saieg, 1980 an Amer et al., 2010). Also 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 1.35.Also Kulkarni et al (2007) demonstrated that immunized groups against NE had significantly fewer chickens with lesions than the non immunized control groups.

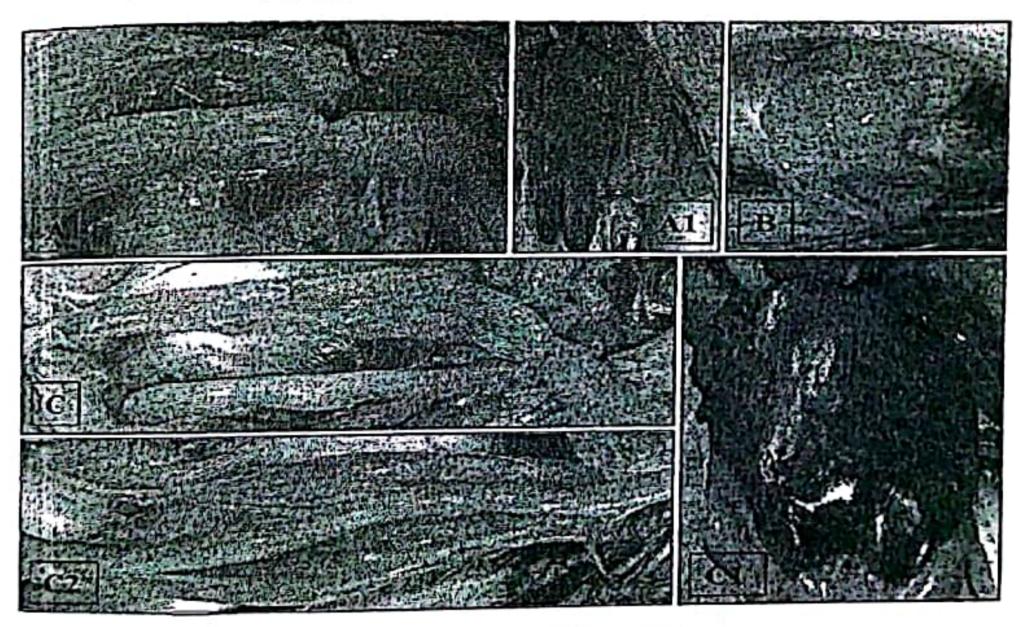
Table (3): Gross pathological lesions score observed in chickens groups given NE vaccine and infected with C. perfringens.

Gr No	organ	Days after infection
1	Intestine Liver	6 9 12 15 0
2	Intestine Liver	0 1+
3	Intestine Liver	1+
4	Intestine Liver	1+ 2+
5	Intestine liver	1+ 1+
6	Intestine liver	1+ 2+ 2+ 1+ 2+
		2+

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 in flammation, 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 12th dpi (plate 3, 3) with focal necrotic area with mononuclear cells infiltration at 15th dpi

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



A: 9 dpi; Haemorrhages in intestinal wall.

B: 12 dpi; Massive necrosis and haemorrhages in intestinal mucosa.

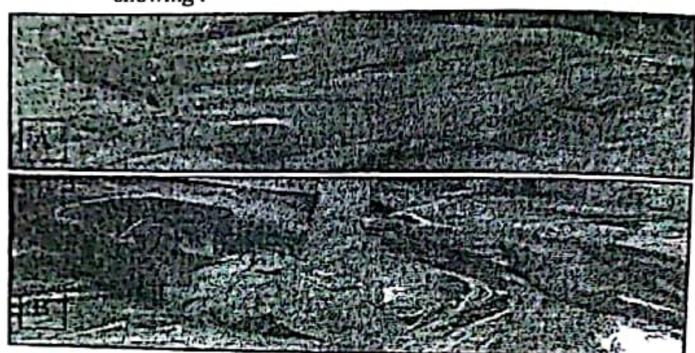
C: 15 dpi; Haemorrhages in intestinal wall.

A1: liver necrosis.

C1: liver necrosis.

C 2: Cecal core.

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.

(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). On the other hand the intestine showed submucosal oedema and congestion at 3-15 dpi (Plate5, 4). 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.

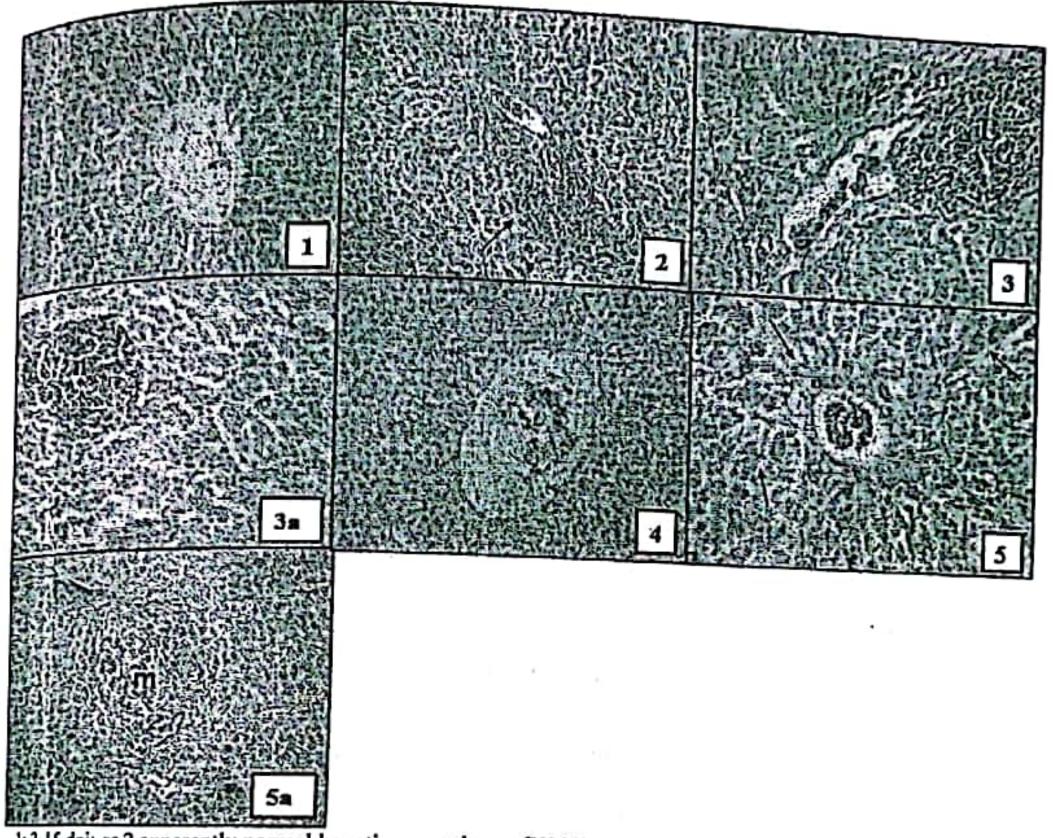
Liver Sections of group 6 were showed dissociation and disorganization of hepatic parenchyma at 3rd dpi (Plate 3, 5); while the focal area of mononuclear cells infiltration was seen at 6-15 dpi (Plate 3, 5a) .At 3-15 dpi (Plate 3, 5) intestine showed mucosal necrosis and leucocytic infiltration. Kwatra and Chaudhury

(1976), Al-Sheikhly and Truscott (1977) and Broussard et al. (1986).

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). Generally ther detewcted lesions in vaccinatexd groups were less severe than control infected nonvaccinated one.

In conclusion: The obtained results indicate that single dose of NE vaccine had role in minimizing the effect of challenge with C. perfringens on performance, gross and histopatrhological lesions. The used NE vaccine had a role in prevention of NE in presence of aflatoxins and/or cocidia vaccines. This findings required more investigations especially in presence of immunosuppressive factors.

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).

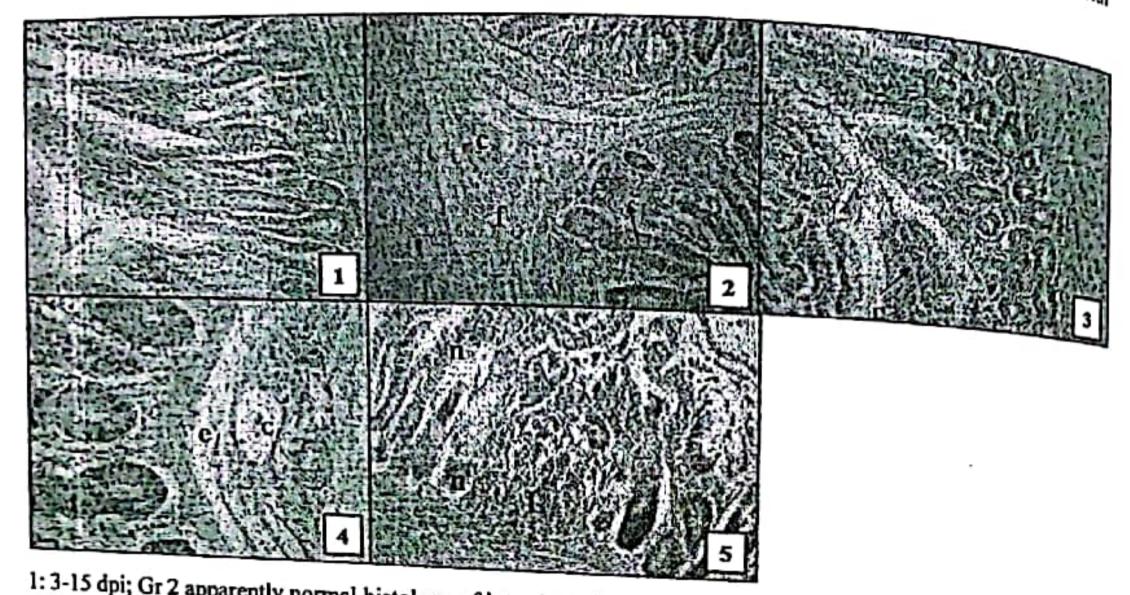
3: 12 dpi; Gr 4 portal leucocytic infiltration (L) (X200).

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).

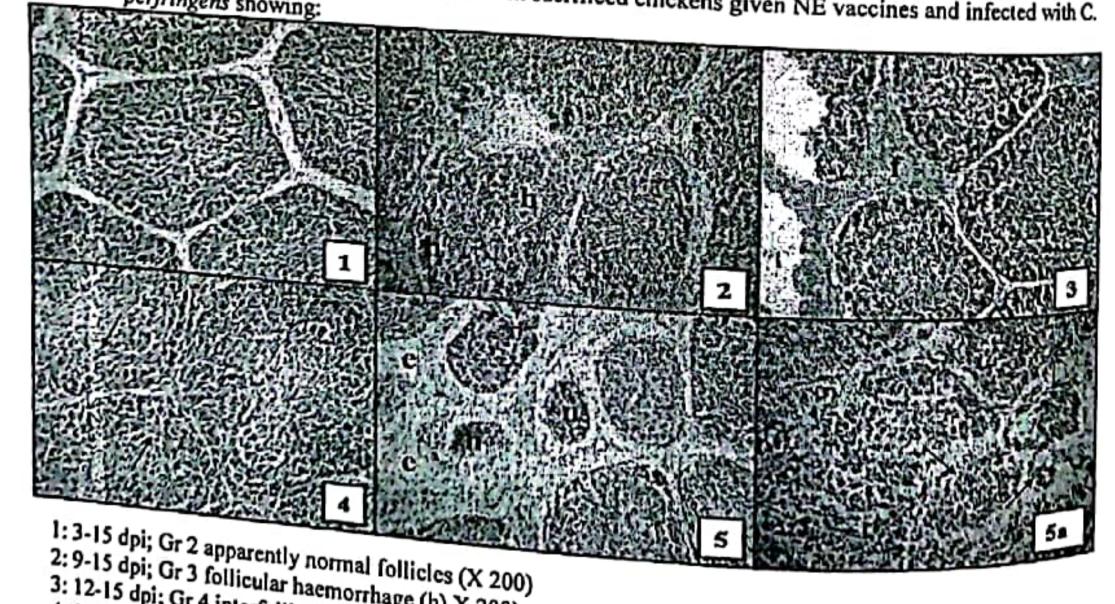
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Plate (4): Intestinal tissue sections stained with H&E in sacrificed chickens given NE vaccines and infected with



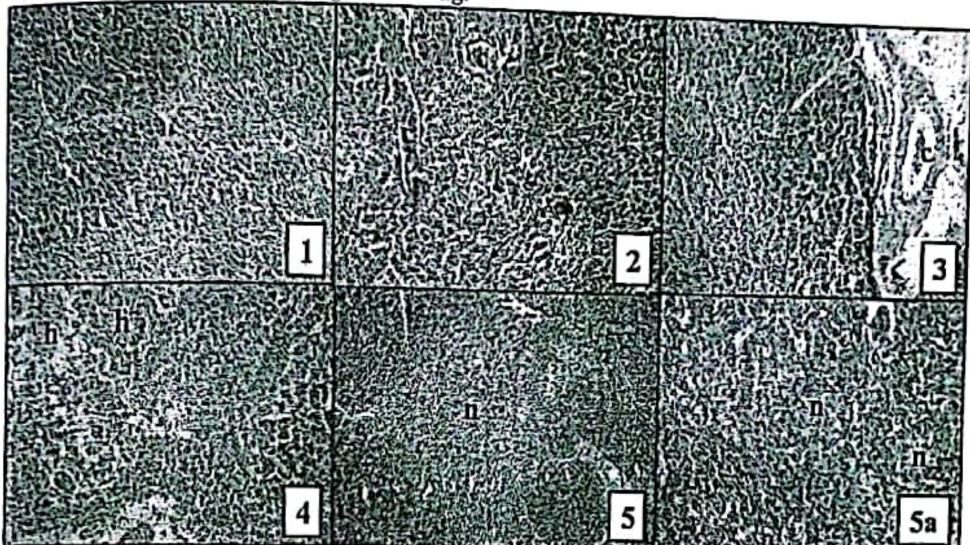
- 1: 3-15 dpi; Gr 2 apparently normal histology of intestinal tissue (X 200).
- 2: 12-15 dpi; Gr 3 mucosal inflammation (arrows) and submucosal fibrosis (F) and congestion (C) (X 200). 3: 12-15 dpi; Gr 4 hemorrhagic mucosa (arrows) (X 200).
- 4: 3-15 dpi; Gr 5 submucosal oedema (e) and congestion (c)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



- 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).
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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).

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التحكم في الاصابة بالتهاب الامعاء التنكرزي في الديوك باستخدام لقاح التحكم في الاصابة بالتهاب الكولسترديومبيرفرنجنس

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استخدام لقاح ميت لالتهاب الامعاء التنكرزي في الوقاية من العدوى الصناعية في وجود العوامل المهيئة للاصابة . اظهرت المجموعات التي أعطيت اللقاح نتائج أفضل من المجموعة الضابطة من حيث الاثارالمرضية والتي تمثلت في انخفاض الاوزان ومعدلات التحويل الغذائي وكذا الأفات المرضية التشريحية والمجهرية .

كان لاعطاء اللقاح اثارا ايجابية واضحة في السبع ايام الاولى من العدوى في المجموعة التي اعطيت لقاح الكوكسيديا كعامل مهيء للمرض تلها المجموع التي اعطيت لقاح الكوكسيديا والسم الفطرى بينما كانت نتائج المجموعة الضابطة غير المحصنة الاقل.

ادى اعطاء لقاح الالتهاب المعوى التنكرزى الى تاخر ظهور الافات التشريحية والنسيجية الى مابعد اليوم السابع ؛ كما لوحظ الاثر الايجابى الواضح للقاح على الافات النسيجية المرضية لغدتى البرسا والثايمث. امكن التعرف على اثارا ايجابية لاستخدام اللقاح المثبط لالتهاب المعى التنكرزي في الدجاج.

استخدام اللقاح يحتاج الى المزيد من الدراسات خاصة في وجود العوامل المهيئة لتحقيق الاستخدام الامثل له.