Necrotic Enteritis in Chickens and Trials for Vaccine Preparation from the Toxins of Isolated *Clostridium Perfringens*

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Abstract

In the present study 305 (72.6%) isolates of *C.perfringens type A* were isolated from liver and intestine of 420 dead broiler chickens with post mortem lesions of NE .In addition to 25 (31.25%) isolates of *C.perfringens type A* were isolated from liver and intestine of 80 dead broiler chickens with no gross lesions of necrotic enteritis (NE). Toxoid vaccine adjuvanted with 20% Aluminum hydroxide gel was prepared from the most toxigenic *C.perfringens* type A strains. Vaccination of two groups of 2 weeks old chicks with 0.25ml and 0.5ml of the prepared toxoid revealed a high antitoxin titer against *C.perfringens type A* (0.5 IU/ml and 0.9 IU/ml respectively). Also vaccination of two groups of chickens 6-8 weeks of age with 0.5ml and one ml of the prepared toxoid revealed a high titer (0.7 IU/ml and 2 IU /ml respectively) .The inclusion of antibacterial feed additives has until now been the major strategy for controlling *clostridium perfringens (C. perfringens)* associated necrotic enteritis (NE) in broilers. These antibacterial feed additives may have bad effect on the consumer as well as drug resistant they develop. So, Immunoprophylaxis seems to be an interesting alternative for the control of necrotic enteritis in chickens.

Introduction

Necrotic enteritis in domestic chickens has been reported from most countries with intensive poultry production. The clinical and subclinical form of necrotic enteritis infection in poultry are caused by C.perfringens type A and to a lesser extent type C (10; 35)

The acute form of the disease leads to increased mortality in the broiler flocks. This can account to 1% losses per day during the last weeks of the rearing periods (23). In subclinical form damage to the intestinal mucosa caused by C.perfringens leads to decreased digestion, absorption and reduced weight gain. (9; 17; 18; 24). Moreover , it is shown that cholangiohepatitis occurs in the subclinical form of

C.perfringens infection lead to an increase in number of condemnation at processing due to these liver lesions (26) Alpha- toxin produced by C.perfringens has been suggested to be a key virulence determinant (33)

Most published reports of NE have involved commercial broiler chickens raised on the floor (31). In addition, the majority of NE outbreaks have been reported in 3 to 5 – week old broiler chicks (15)

In several countries the poultry industry agreed upon stopping the use of antibacterial growth promoters whereas anticoccidial feed additives are still used. However public opinion against feed additives is growing which increases the interest for development of alternative control strategies (15). The incidence of C.Perfringens-associated NE in poultry has increased in countries that stopped using antibiotic growth promoters (20)

No satisfactory non antibiotic measures against C.perfringens in poultry have been indentified, anticlostidial vaccinations may become an opinion in the near future, but whether such vaccination can fully replace in- feed antibiotics remains to be seen (22)

A vaccine for NE of chicken would reduce the need to prevent or treat the disease in broiler chickens with antibacterial drugs (38). Allover the world, the available literatures dealing with vaccination of poultry with clostridial vaccines are relatively scanty (27)

The present study is planned to clear the incidence of C. perfringens in broiler chickens suffering from necrotic enteritis and trial for preparation of anticlostridial vaccine from the isolated strains.

Materials and Methods:

Samples

Five hundreds dead broiler chickens (20-43 days of age) were collected from El-Kalyoubia, EL-Minofyia and El-Gharbia provinces. These birds suspected to have necrotic enteritis. Livers and intestinal samples were taken for anaerobic cultivation and identification (34; *41)*. Intestinal contents were centrifuged and the supernatant checked for *C. perfringens* toxins *(7)*.

Typing of the isolated strains and detection of toxins in the intestinal contents were done by using toxin neutralization test in albino guinea pigs, dermonecrotic reaction (36)

Vaccine preparation

Three batches of vaccines were prepared from the most toxigenic isolated strains of *C. perfringens* type A (1). Toxoid was clarified and concentrated by ultrafiltration system.(Millipore Corportaion, Bedford Massachusetts, 01730,USA) Aluminum hydroxide gel was added to the concentrated toxoid in a percentage of 20% of toxoid volume as an adjuvant *(8)* .Tests for purity and safety of the vaccine batches were carried out (4)

Birds

A total of 120 two weeks old and 6-8 weeks old Hubbard chicks were used for the dose determination of the prepared vaccine .Another 170 of 6-8 weeks of age Hubbard chicks were used for the evaluation of the three prepared batches of the vaccine

Dose determination for different ages of chickens

Two groups each of 30 chicks aged 2 weeks were vaccinated subcutaneously at the neck region with two doses of the vaccine with 3-4 weeks interval. The dose in first group was 0.25 ml and in the second one was 0.5 ml. Another two groups each of 30 chicks aged 6-8 weeks were vaccinated with two doses of the vaccine with 3-4 weeks interval. First group received o.5 ml and the second group received one ml dose

Evaluation of the prepared batches of vaccine

Three groups of broiler chickens aged 6-8 weeks each of 50 chicks were used for evaluation of the three prepared vaccine batches .Each chick received two doses of 1 ml subcutaneously at the neck region ,3-4 weeks apart .Another fourth group of 20 chicks was used as unvaccinated control group. Blood samples were collected from all groups, two weeks after the second dose, sera of each group were pooled and tested for determining the alpha antitoxin titer of *C .perfringens type*

A expressed as international unit pre ml (I.U/ml) by using serum neutralization test (11)

Experimental animals

a-Albino guinea pigs

Local breeds of albino guinea pigs weighting 350-450 gms were used for dermonecrotic reactions as well as typing of *C perfringens* isolates

b-Swiss mice

Mice with an average weight of 15-25 gms were used for the determination of toxigenicity and typing of *C.perfringens* isolates

Results and Discussion

Necrotic enteritis is an acute enterotoxaemia; the clinical illness is usually much shortened often the only signs are a sudden increase in mortality *(28) Five* hundred samples were taken from dead broilers with necrotic enteritis suspected disease, from three provinces in Egypt (El-Kalyoubia, EL-Minofyia and El-Gharbia). The case history of these birds was acute death, with high mortality rate, depression, dehydration, ruffled feathers, diarrhea and decreased feed consumption. These clinical signs were reported previously *(2; 3; 13;* 16*)*. Necropsy examination of these birds revealed that there is 420 have intestinal necrosis and congestion of livers. Also there was enlarged gas filled intestines, the mucosal surface of which had the Turkish towel, in addition to severe congestion in liver and kidneys. These results agreed with a previous result (5*)* .Our findings support those obtained by other investigation *(12)* which found an increase in the mortality rate and characteristic extensive necrosis of the small intestinal mucosa in dead birds with necrotic enteritis

In the present study it was found that all isolates from dead birds with or without necrotic enteritis were *C.perfringens type A* only. These results run parallel with that obtained previously (18*)* which stated that in a Norwegian survey of *C.perfringens* from broilers and C.apercaillis with

and without necrotic enteritis, all the 192 isolates were *C.perfringens typeA*. Also the present results agreed with other *(25)* which indicated that NE of chickens is caused by *C.perfrigens type A* in almost cases under natural conditions as *C.perfringens type A* is the strongest producer of α-Toxin .On the bases of the present results and others (25), α-Toxin is the single major toxin of *C.perfringens* , which is responsible for necrotic enteritis of chickens

C.perfringens type A was isolated from 305 out of 420 (72.6%) intestinal samples having intestinal necrosis, whereas it was isolated from 25 samples out of 80 (31.25%) which haven't intestinal necrosis as seen in table 1. Our results agreed with a previous result (24*)* which showed that semi-quantative examination of ileal contents showed abundance of *C.perfringens* among 37 of 38 (97%) tested birds with intestinal necrosis as compared with 18 of 90 (20%) among birds without intestinal necrosis. Also, *it has been reported (15)* that NE usually associated with high numbers of the intestinal bacterium *C.perfringens.* In addition, anaerobic organisms included mostly *C.perfringens* (*29*). (C.perfringens *type A* was the main isolate followed by type D then type C) were recovered from 93 out of 155 (60%) intestinal samples from chickens with necrotic enteritis.

The intestinal filtrate of the 420 samples having intestinal necrosis was positive for alpha toxins of *C.perfringens type A*, although 305 samples only have *C.perfringens type A* organisms. This may be due to previous treatment with antibiotics .While 44 out of 80 (55%) samples which haven't intestinal necrosis were positive for alpha toxins .These results agreed with that observed previously (6*)* where alpha toxins of *C.perfringens type A* was demonstrated in the intestinal contents of 48 dead rabbits, from which 20 *C.perfringens* isolates obtained only

Research should focus on creating new protective measures against the disease .Vaccination of poultry against *C.perfringens* is a seriously under research matter, despite of the known protective effects in other animal species *(20)*

In this study, the results of vaccination of chicks (2 weeks old) with different doses of the prepared vaccine resulted in a high titer in the group vaccinated with 0.5 ml of the vaccine (9 IU/ml) compared with

those vaccinated 0.25ml (0.5IU/ml). In addition, when older chickens (6-8 week old) vaccinated with 1 ml of the vaccine resulted in a high titer (2 IU/ml) than those vaccinated with a dose of 0.5ml (0.7 IU/ml) as seen in table (2). Our results differed, to some extent, with a previous study (27) which used a dose of 0.25 ml for vaccination of breeder hens. Based on the results of a pilot study, testing different doses of the toxoid prepared vaccine, immune responses were largely independent of dose levels varying from 0.25 to one ml (unpublished results).. Also, vaccination of adult rabbits with different doses of the prepared vaccine (*8*) resulted in high titer in group vaccinated with 2 ml of the vaccine (1.5 IU/ml) compared to those vaccinated with one ml (0.4 IU/ml) , but there was no difference in antitoxin titer in sera of kits (4-6 weeks) either vaccinated with 2 or 1 ml of the vaccine (1 IU/ml).

Table (3) shows the antibody titers against alpha toxins of *C.perfringens* type A in vaccinated chickens which determined by using serum neutralization test (11). *I*t was found that all batches of the vaccine produce good antibody titers which can protect chicks from the disease. It is known indeed that flocks with high titers of maternal antibodies against alpha toxins had a lower mortality during the production period than flocks with low titers *(15)*. Also, vaccination of broilers with candidate vaccine, based on *C.perfringens* type A and type C toxoid, gave promising results in respect of protection against subclinical necrotic enteritis. (*27)*. Specific antibodies against alpha toxins of *C.perfringens* might protect broilers from NE, as seen in diseases caused by various *C.perfringens* toxin types in other species. *(32; 37)*. A vaccine was prepared from alpha toxins of *C. perfringens type A* for rabbit (*6), where the* vaccinated rabbits have good immune response (1-1.5 IU/ml). Rabbits can be protected when the blood level of circulating alpha-antitoxin of *C. perfringens type A* is raised to 0.1 Iu/ml (39; *40*). .In addition, *rabbits* produced antitoxin titer in a range of 0.5-4 IU/ml to alpha toxin of *C.perfringens type A* vaccine *(30; 14; 21)*

More investigation is needed to evaluate the efficacy of this vaccine in the field .We recommend to produce the vaccine and use it in control of necrotic enteritis in broilers in Egypt.

Table (1): Types of *C.perfringens* strains isolated from liver and intestinal content and types of toxins determined in the intestinal filtrate from dead broilkers

Province	El-Kalubia	El-Menofia	El-Gharbia	Total
Total No. of samples	200	180	120	500
No. of samples with Intestinal necrosis	180	150	90	420
C.perfringes isolated From necrotic intestine	120 (66.60)	105 (70.00)	80 (88.80)	305 (72.60)
Intestinal filtrates with +ve alpha toxins	180 (100.00)	150 (100.00)	90 (100.00)	420 (100.00)
No. of samples without Intestinal necrosis	20	30	30	80
C.perfringes isolated From non-necrotic intestine	8 (40.00)	9 (30.00)	8 (26.60)	25 (31.25)
Intestinal filtrates with +ve alpha toxins	12 (60.00)	17 (56.60)	15 (50.00)	44 (55.00)

Table 2 : Determination of suitable dose for different ages of chicks

Age	No of birds	Dose /ml	Titer (iu / ml)
2weeks	30	0.25	0.5
	30	0.50	0.9
6-8weeks	30	0.50	0.7
	30	1.00	2.0

iu / ml = international unite / milliliter

Table 3 : Alpha antitoxin titers in chicks vaccinated with different batches of vaccine

Group no	No of birds	Vaccine batch	Antitoxin titer IU/ml
Group 1	50	I	2
Group 2	50	II	3.5
Group 3	50	III	5
Group 4	20	Unvaccinated	-Ve

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التنكرز المعوي في الدجاج ومحاولات لتحضير لقاح لسموم الكلوستيريا بيرفرنجينز المعزول

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في هذه الدراسة تم عزل ٣٥٠ عترة من الكلوستيريا بيرفرنجينز نوع (ا) من ٤٢٠ حالة دجاج تعاني من تنكرز معوي بنسبة ٧٢%، كذلك تم عزل ٢٥ عترة من نفس الميكروب من ٨٠ حالة غير واضح عليها تنكرز معوي، تم عمل لقاح من سموم ميكروب الكلوستيريا بيرفرنجينز نوع (أ) وتم تحميله بواسطة هيدروكسيد الالومنيوم بنسبة ٢٠%،

تم تحصين مجموعتين من الكتاكيت بهذا اللقاح بجرعتين مختلفتين (٠,٢٥، ٠,٥٠ مللي) تحت الجلد، ووجد تكوين اجسام مضادة بنسبة عالية (٠,٥، ٠,٩ وحدة دولية بالتتابع)،

كذلك تم تحصين مجموعتين من الدجاج عمر ٦-٨ اسبوع بجرعتين مختلفتين (٠,٥، ١,٠ مللي) تحت الجلد، ووجد ايضا تكوين اجسام مناعية بنسب عالية (٠,٧، ٢,٠ وحدة دولية بالتتابع)،

وحتي الان يوجد استخدام لمضادات البكتيريا في العاف لعلاج التنكرز المعوي، وهذ يضر بصحة الانسان، كذلك تتكون عترات مقاومة للعالاج ، لذلك فان استخدام اللقاح لمقاومة التنكر المكعوي يعتبر وسيلة جيدة بديلة لاستخدام مضادات البكتيريا.