Trials for vaccination by clostridial perfringens in broiler

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Abstract

C. perfringens type A and C were isolated and fully identified serology and PCR . these starins used in preparation of vaccines: *C. perfringens* toxoid type C and mixed toxoid A&C. *C. perfringens* bacterin A, C and mixed bacterins A& Broiler chicken were divided into 7 groups, 6 groups for vaccinations and last o as control. Broiler chicks were vaccinated subcutaneously at 7 and 21 days age , followed 7 days later challenge with *Clostridium perfringens* type A&C. N vaccinated birds challenged with *C. perfringens* developed mean of score lesio of (1.88) while mean of score lesions in vaccinated groups with toxoid of *perfringens* type A, toxoid of *C. perfringens* type C and mixed toxoid vacci prepared from type A and C were 0.173, 0.231 and 0.123 respectively. Wh immunization of broiler chickens with killed vaccine (bacterin) revealed th mean of score lesions of non vaccinated chickens was (2.223) although, vaccinated chickens with *C. perfringens* type A bacterin, *C. perfringens* type bacterin and mixed bacterin vaccine of type A&C were 0.17, 0.216 and 0.1 respectively. Vaccination produced antibody response which measured w ELISA and revealed that antibody titer in vaccinated birds with (toxoid a bacterin) were higher than the antibody titers in non vaccinated birds. The results suggest that vaccination of the broiler chicken with (toxoid and bacter may protect the birds from challenge with *C. perfringens* and may serve effective vaccine.

Introduction

Clostridiosis in broiler flocks is nowadays controlled by the routine use antibiotic feed additive (Ficken and Wages, 1997). Antibiotic feed additive cau drug resistance and residual in meat. During recent years, there is an increas focus in the poultry industry on meat production without such additiv (Lowlander *et al.*, 2003 and 2004).Clostridial vaccines are recommended prophylaxis against diseases caused by Clostridia (Prukner *et al.*, 1995).Seve efforts have been made for preparing of Clostridial vaccines by Gadalla *et* (1969 and 1974) and Farrag *et al.*, (1988).Allover the world, the availa literatures belonging to the vaccination of poultry with clostridial vaccines relatively scanty (David, 1973; John et *al.*, 1982 and Lovland *et al.*, 2004).

From all mentioned before necrotic enteritis (NE) is an economically import enteric disease of chickens caused by *C. perfringens*. Although vaccination off an alternative approach to antimicrobial drugs in control of the disease, littl known about immunity to (NE). However, there is the suggestion that the alp toxin, phospholipase C exoenzyme, is an important immunogen (Lovland *et* 2004 and Stevens *et al.,* 2004). A recent study also, showed that it is possibl immunize broiler chickens successfully against NE. The immunizing ability v associated with virulent but not with avirulent strains and some alpha-toxin-mi mutants also successfully immunized chickens against infection Thompson *et*

(2006)Therefore, this recent study was planned to study the preparation of tox and killed vaccines of the commonly isolated C. perfringens types "A" and .Studying the efficacy of the prepared vaccines in Boiler chickens was detec by: Evaluation of antibodies against C. perfringens type "A" and type "C" by us the Enzyme Linked Immunosorbent Assay (ELISA) assay.

Material and methods

C. *perfringens* strains type A and C isolated were isolated from local chicken. isolates were fully identified morphologically, cultural characters, biochemical a confirmed by dermonecrotic reaction test in Guinea pigs and diagnosed by P(Experimental birds and animals include: Mice weighing 20-25g, were used safety tests, determination of the MLD of the toxin and 200 one day old bro chicks (Ross-308) were obtained from the EL-Wadi company for chicl production at Sadat City. Balanced feeding ration was obtained from priv company. these strains were inoculated on cooked meat medium for enrichm purification of the clostridia strains. Sheep blood agar medium and Pept(medium (Roberts et al., 1970).It was manipulated for the production of to: Formalin in the concentration of 0.3% was added to each clostridial toxin (A and kept in incubator for 72 hour with checking daily. After that solutions w centrifuged at 5000 rpm for 5 minutes, the clear supernatant used as toxoid (A and dead cell sediment used as Bactrian (A-C). Complete detoxification v regarded when 0.2ml of toxoid was injected I/V into mice with the survival of mice after 8 hours Gadalla *et al.*, 1969. Phenol was used as an antibacte agent. It was prepared as phenol saline and used for preservation of the tox vaccines. Potassium aluminum sulphate (Alum) used as adjuvant.

Sterility tests were done by one ml from the prepared toxoid was transferred i two tubes, which were, fluid thioglycolate, nutrient agar slope, both w incubated aerobically at 37°C. In the meantime one ml of the prepared toxoid v incorporated into cooked meat broth and incubated anaerobically at 37°C. T test tubes of Sabourauds agar were also inoculated, one incubated at 37°C a the other incubated at room temperature. All tubes were kept under observat for ten days for bacterial or even fungal growth. The vaccine is conside passable if no growth appeared in any of the inoculated media. Safety te preliminary tests were made by injecting 1ml of each /toxoid I/V into five mice. mice survived without showing symptoms of disease. The final product v precipitated with 1% potassium alum; phenol was added as preservative in a fi concentration of 0.4% and pH readjusted to 6.5.
Immunization of broiler chicken with toxoid "A" ,"C" and Bactrian" A" , According to Kulkarni et al., (2007):Eight groups of broiler chicken (Ross 308) the age of one day. The total group of chicken were divided as (3 groups ea one consisted of 25 chicken vaccinated by toxoid "A, C and AC) respectively a another (3group each one consisted of 25 chicken were vaccinated with *perfringens* bacerian type A, C and AC respectively. These six groups w vaccinated by two doses at age 7days and 21days . The dosage of vaccinat was (0.5ml s/c). the seventh group taken one dosage only. This group v contain 15 chicken [5 chicken were vaccinated with toxoid A, 5 chicken w

vaccinated with Bactrian A and last 5 chicken were vaccinated with toxoid AC vaccinated by one dose (0.5ml s/c) and the eighth non-vaccinated group serve as control group. This illustrated in table (1).

Blood samples: Three ml blood was taken in a sterile wesserman tube fo separation of serum, which was stored at -20°C in an ependorf for th measurement of the antibody titers by I-ELISA technique. The collection of bloc was carried out before the 1st dose of vaccination and 3 times. One time after fir dose of vaccination by 2 weeks interval and two times after booster dose after week interval

Challenge of Immunized chicken by C. Perfringens type A and C according t Kulkarni et al., (2007):The groups which previously immunized as mentione before were kept under observation and protected by vaccination prograi against viral diseases such as Newcastle and Gumboro virus diseases, all th birds groups challenged with virulent strains of *C. perfringens* type A and C grown in cooked meat medium for 24 h at 37°C. Fluid thioglycolate medium wa then inoculated with a 3% (vol /vol) inoculum from the *C. perfringens*- infecte cooked meat and incubated at 37°C for 24 h. The growth at 24 h was 8.24 ± 0.0 *C. Perfringens* log 10 CFU /ml. The inoculated fluid thioglycollate medium wa then mixed with feed at a ratio of 2: 1(vol/w). The inoculated feed was fresh prepared twice per day and feed to chicken that were fasted for 20 h prior challenge for 3 successive days. Another route of infection used in th experiment by adding 5ml from infected thioglycolate medium which wa contained 8.24 ± 0.09 *C. Perfringens* log 10 in drinking water for successivedays .(Material and Procedure of ELISA according to (Harlow ar Lane, 1988.

Results

Intestinal lesion scores of birds immunized with one injection s/c with toxoid bacterins type A and mixed toxoid types A,C and then challenge with *perfringens* type A and C. Experiments No. (3), the table (4) showed that TI birds immunized with toxoid A, Bacterin A and toxoid A and C by one injection s 0.5 ml from each type . The immunization occurs by one dose only and after o week of immunization the birds were been challenged with *C. perfringens* type and C orally for 3 days. The results refers to mean of score lesions of bir immunized with one dose of vaccines is higher than mean of score lesions birds immunized with two doses of vaccine. The toxoid A, bacterin A and toxoid and C offered significant immunity to the immunized broiler chickens but, t bacterin A give higher level of protection to the birds.

Results of ELISA test for evaluation of *C. perfringens* antibody levels vaccinated chickens:

1. The antibody levels of the serum samples collected from broiler chick immunized with toxoid of C. perfringens type A, type C, and mixed toxoid typ A,C at different time pre and post immunization :

Table (5) showed the results of broiler chickens those vaccinated with toxoid C. perfringens type "A" the O.D. values for the serum collected from broi chickens pre-vaccination (zero time) and post vaccination at 2, 3, and 4 wee were 0.446, 0.648, 0.875 and 0.838 respectively .

0.443, 0.689 and 0.556 at zero time and after 2, 3, and 4 weeks respectively the other hand O.D. values for the serum collected from broiler chicl vaccinated with toxoid of *C. perfringens* type "C" were 0.493 at zero time 0.556, 0.765 and 0.861 at 2, 3, and 4 weeks respectively. The third grou chicken vaccinated with mixed toxoid (A and C) showed that the following values were 0.502 at zero time and 1.019, 0.885 and 1.015 at 2, 3, and 4 we post vaccination respectively. From the results reported in table (5) we not that, there are gradual increase in the antibody levels expressed by gra increase in the O.D.

As compared with non vaccinated group it was clear that the O.D. values 0.405,

values for the serum collected from broiler vaccinated with toxoid o *perfringens* type "A", "C" and mixed toxoid A and C in comparison with control non vaccinated broilers. The antibody levels of the serum sam collected from broiler chicken immunized with bacterin of *C. perfringens* typ type C, and mixed bacterin types A,C at different time pre and post immuniz : In table (6) results of broiler chickens that vaccinated with bacterin o *perfringens* type "A" the O.D. values for the serum collected from broiler chicl pre-vaccination (zero time) and post vaccination at 2, 3, and 4 weeks were 0. 0.754, 0.803 and 0.911 respectively. The O.D. values for the serum colle broiler chickens vaccinated with bacterin "C" at zero time and at 2, 3, a weeks post vaccination were 0.523, 0.741, 1.125 and 0.417 respectively. O.D. values for the serum collected broiler chickens vaccinated with m bacterin of *C. perfringens* type "A" and "C" at zero time and post vaccination 3, and 4 weeks were 0.220, 0.807, 1.190 and 0.932 respectively. While The values for the serum collected from control group at zero time and at 2, 3, a weeks post vaccination were 0.502, 0.604, 0.606 and 0.644 respectively.

The antibody levels of the serum samples collected from broiler chi immunized with toxoid A, bacterin A, and mixed toxoid types A,C at different pre and post immunization this group immunized with one dose of vaccine:

Table (7) showed that The O.D. values of the serum collected from br chickens immunized with one dose of vaccine of toxoid A, bacterin A and m toxoid A and C. For toxoid "A" at zero time before vaccination and at 2,3 w post vaccination the O.D. values were 0.446, 0.637 and 0.503 respectively. bacterin A O.D. values were 0.646 at zero time and 0.764 at 2 weeks vaccination and 0.493 at 3 weeks post vaccination . The O.D. values of serum collected from chickens vaccinated by toxoid A,C were 0.502, 0.905 0.624 respectively. While the O.D. values of control group were 0.425, 0.405 0.302 respectively.

Discussion

Protection against poultry NE by vaccination with *Clostridium perfringens* ty toxoid has been controversial. On the one hand, Lovland *et al.,* (2004) vaccin hence with crude "type A" or type "C" toxoid (containing CPA) and progeny protected against sub clinical NE. However, this provides little information a the role of an anti-CPA response in protection; these toxoids contained n other antigens against which the birds may have produced a protective imm

response. On the other hand, birds inoculated with a CPA mutant were protect against subsequent challenge with the virulent isolate (Thompson *et al.*, 200 Songer (1997) used two vaccines for maternal immunization contained differe inactivated toxins. In type "A" toxoid, alpha-toxin of *C. perfringens* is the only *perfringens* major toxin present while, type "C" toxoid contains both beta-to and alpha-toxin. In both toxoids several *C. perfringens* minor toxins may present but the exact composition in this respect is not known for the toxoi used . In the last few years, large numbers of the Enzyme Linked Immunosorbe Assay (ELISA) for toxin and antitoxin were developed .

In table (12) in immunization experiment No. 1 toxoid A, toxoid C and mix vaccine, toxoid A&C offered significant protection against challenge . From t other hand mixed toxoid A&C vaccine give the greatest protection while, toxoid was the lowest protection . When compare between table (12) which show lesi scores of vaccinated broilers with toxoid (A,C and mixed A&C) with table (1 which show the antibody levels of the serum samples collected from broi chickens vaccinated with toxoids found that gradual increase in the O.D. valu for the sera collected from broilers vaccinated with the toxoid of *C. perfringe type "A"* and type "C" and mixed types "A and C" in comparison with control n vaccinated broilers.

Also, it is important to note that the levels of antibodies were greater in serum broilers vaccinated with the mixed toxoids of *C. perfringens* type "A and C" th the antibody levels in serum of broiler chickens vaccinated with toxoid "A" a toxoid "C" respectively. This increase continued up to the end of observati period. The immunization dependant increase in OD values for serum can attributed to the increased production of immunoglobulin by immunocompete cells. These results agree with those reported by Ginter *et al.*, (1996); Ficken a Wages (1997); Justin *et al.*, (2002); Lovland *et al.*, (2004); and Thompson *et a* (2006), who induced successful immunization in broilers against *C. perfringe* toxoids "A" and "C" . in addition, better responses may result from using different adjuvant, higher doses of immunogen or alternate routes of delivery. this study where vaccinated S/C, which typically generates a strong IgG (Ig response. These results like those reported by (Cooper *et al.*, 2008); IgG (Ig has a key role in the immune response to NE in broiler chickens (Lovland *al.*,2003and 2004) and for this reason, we didn't examine the IgA response of t bird. Stimulation of strong IgA response by mucosal immunization might provi better protection.Alpha toxin has been considered the most important pathoger factor in the development of NE when type "A" is the causative agent (Ficken a Wages, 1997).

It was surprising to find that toxoid "C" vaccine induced a slightly lower Ig antibody level than the IgG antibody level against the toxoid "A" vaccine. The results can be explained for the differences in alpha toxin structure of type " and "C" (Ginter *et al.*,1996 and Justin *et al.*, 2002). The configuration of comm antigens may also have been differed in the two toxoids, due to different levels configuration changes in the formaldehyde inactivation procedures (Lovland *al.*, 2004)It remain to note that the result of this work is agreed with Cooper *et a* (2008); and disagreed with Keyburn *et al.*, (2006) who stated that CPA w suggested to be unnecessary attributed in pathogenesis of NE. it may be th other attributes are required for establishment of the infection and inhibition

lesion development, and that CPA adds the severity of the disease. Table (
and fig (4) results of using *C. perfringens* bacterin A,C and mixed bacterin A
offered protection against challenge but the mixed bacterin type A,C offer
greatest protection.
the increasing mean lesions scores for the control group compared to those
the birds in experiments 1,2 appeared to reflect the increasing of immunity
immunized groups than un immunized control groups. These results agreed w
the results of Kulkerni *et al.*, 2006 and Cooper *et al.*, 2008. When compare
result of intestinal lesion scores of birds vaccinated with bacterin A,C and mix
bacterin A&C with the table (16) which show the antibody levels of the ser
samples collected from broiler chickens immunized with bacterin of
perfringens type A, type C, and mixed bacterin types A& C found that
antibody levels in the serum immunized chicken with mixed bacterin A&C
relatively higher than the antibody level in chicken serum vaccinated with b
bacterin A and bacterin C. In table (17) fig(5) When compared between antibo
levels of the chickens immunized with toxoid and bacterin found that bacte
vaccine give antibody leve's were relatively higher than the antibody levels
toxoid vaccine. It is clear that immunization of broiler chickens with toxo
vaccines (A, C and mixed A&C) and with bacterin vaccines (A, B and mixed A&
were adequate to induce antibodies sufficient for chickens to tolerate the act
challenge with *C. perfringens* type "A" and "C" . The results of this study
agreed with the recent studies of Thompathon *et al.*, (2006); Kulkerni *et*
(2006) (2007) and Cooper *et al.*, 2008. In table (15) (16) in present study
noticed that antibody levels in control non immunized chickens were slightly h
this due maternal antibodies which may interfere with the immune response
vaccine .
This study used commercial birds as specific pathogen free (SPF) but birds w
not free from *C. perfringens* maternal antibodies that passed into yolk
evidenced , by high level of antibody titer in newly hatched chickens. T
explained by Cooper *et al.*, (2008) who used recombinant alpha toxin
immunization of broiler chicken.Table (18) in experiment No. 3 trails
immunization of broiler chicken with toxoid A, bacterin A and bacterin A and A
by one dose of the vaccine only not boostered by second dose .
The mean of scores lesions of chickens which vaccinated by one dose of vacc
was higher than the mean of scores lesions of chickens which vaccinated by
doses of vaccine (Ficken and Wages, 1997 and Lovland *etal.*,2004).

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نص العربي

تم تحضير نوعان من التحصينات من ميكروب الكلوستريديم بيرفيرنجينس نوع (أ) و (سى) حيث تم يرهما من السم والبكتيريا الميتة باضافة الفورمالين. وقد تم تحديد الجرعة المميتة للسم المحضر من ستريديم بيرفيرنجينس لكل نوع على حدا

م إعطاء اللقاحات إلى دجاج التسمين عند عمر ٧ و٢١ يوم وبعد أسبوع بتعريض الدجاج المحصن للعدوى روب الكلوستريديم بيرفيرنجينس وقد تم حساب متوسط الإصابة في مجموعات الدجاج المختلفة بطريقة مربع الإحصائية وتجميع عينات الدم وذلك لقياس نسبة الأجسام المضادة في عينات السيرم باستخدام اختبار الاليزا أن نسبة الأجسام المضادة في السيرم المأخوذ من عينات دم دجاج التسمين المحصن بلقاح الكلوستريديم رنجينس من النوع (أ) سواء هو محضر من السم أو من البكتيريا الميتة أكثر من نسبة الأجسام المضادة في دم ج المحصن من لقاح الكلوستريديم بيرفيرنجينس نوع (سى) سواء كان القاح محضر من سم أو بكتريا ميتة.

د استخدام اللقاح المختلط من نوع الكلوستريديم بيرفيرنجينس من نوع (أ) و (سى) سواء كان اللقاح محضر من و البكتريا الميتة وجد أن هناك تزايد في نسبة الأجسام المضادة معبرا عنها بتزايد نتائج الاليزا وقلة متوسط ابة في الدجاج.

ا سبق نستنتج أن اللقاح المحضر من الكلوستريديم بيرفيرنجينس من سموم ا نواع (أ) و (سى) او من البكتيريا ة امن ويمكن استخدامه كوقاية لدجاج التسمين من الإصابة بمرض التكرز الامعائى.

Table (1) Summery of experimental design:

Immunization group	Types of vaccine	Dosage (ml) of vaccine/bird and Frequency of administration	Time of oral challe (feed & water)
Group 1	Toxoid A	0.5 ml s/c two times, at 7 & 21 days of age	3 days after sec dose
Group 2	Toxoid C	0.5 ml s/c two times, at 7 & 21 days of age	3 days after sec dose
Group 3	Toxoid A&C	0.5 ml s/c two times, at 7 & 21 days of age	3 days after sec dose
Group 4	Bacterin A	0.5 ml s/c two times, at 7 & 21 days of age	3 days after sec dose
Group 5	Bacterin C	0.5 ml s/c two times, at 7 & 21 days of age	3 days after sec dose
Group 6	Bacterin A&C	0.5 ml s/c two times, at 7 & 21 days of age	3 days after sec dose
Group 7 Sub group a Sub group b Sub group c	 Toxoid A Bacterin A Toxoid A&C	0.5 ml s/c once at 7 day of age	3 days after first dos
Group 8	control	–	3 days

2. Intestinal lesion scores of birds immunized with two injections s/c with *perfringens* toxoid type A, toxoid type C, and mixed toxoid type A,C and th challenge with *C. perfringens* type A and C.

Table (2): The protection effect of *C. perfringens* toxoid A, toxoid C and toxoic & C

Type of vaccine	No. of chicken	No. of chickens with the following lesion scores						Mean of numb of chickens
		0	+1	+2	+3	+4	+5	
Toxoid A[a]	25	19	5	1	0	0	0	0.173
Toxoid C[a]	25	17	6	2	1	0	0	0.231
Toxoid A and C[a]	25	21	4	0	0	0	0	0.123
Control	15	2	7	5	1	0	0	1.88

[a] :Immunized groups that had significantly fewer chickens with lesions specia using toxoid including both combined C. perfringens type A & C followed by perfringens type A than the unimmunized control group (Fisher's test, p ≤0.05).

Table (3): The protection effect of *C. perfringens* bacterin A, bacterin C and bacterins A & C:

Type of vaccine	No. of chicken	No. of chickens with the following lesion scores						Mean of number of chickens
		0	+1	+2	+3	+4	+5	
Bacterin A[a]	25	20	4	1	0	0	0	0.17
Bacterin C[a]	25	18	4	2	1	0	0	0.216
Bacterin A and C[a]	25	22	2	1	0	0	0	0.146
Control	15	3	4	5	3	0	0	2.223

[a] : Immunized groups that had significantly fewer chickens with lesions specially using toxoid including both combined C. perfringens type A & C followed by C perfringens type A than the non immunized control group (Fisher's test, $p \leq 0.05$).

Table (4):The protection effect of one injection of toxoid A, bacterin A, toxoid A &C:

Type of vaccine	No. of chicken	No. of chickens with the following lesion scores						Mean of number of chickens
		0	+1	+2	+3	+4	+5	
Toxoid A	5	1	2	1	1	0	0	0. 712
Bacterin A	5	2	2	1	1	0	0	0.643
Txoid A and C	5	3	1	1	0	0	0	0.432
Control	5	0	2	2	1	0	0	0.865

* Immunized groups that had significantly fewer chickens with lesions specially using toxoid including both combined C. perfringens type A & C followed by C perfringens type A than the non immunized control group (Fisher's test, $p \leq 0.05$).

Table (5): The antibody levels of the serum samples collected from broiler chicken immunized with toxoids of C. perfringens type A, C and A&C:

Time of serum collection	The optical density (O.D) of serum of broiler chicken			
	Immunized with toxoid A	Immunized with toxoid C	Immunized with toxoid A&C	Nonimmunized control group.
Zero time	0.446	0.493	0.502	0.405
2 week post immunization	0.648	0.556	1.019	0.443

3 week post immunization	0.875	0.765	0.885	0.689
4 week post immunization	0.838	0.861	1.015	0.556

Table(6): Antibody levels of broiler chicken immunized with C. perfringens kill bacterin type A, C, and bivalent A & C (two doses of vaccine

Time of serum collection	The optical density (O.D) of serum of broiler			
	Immunized with toxoid A	Immunized with bacterin A	Immunized with toxoid A &C	Nonimmunize control group
Zero time	0.446	0.646	0.502	0.425
2 week post immunization	0.637	0.764	0.905	0.405
3 week post immunization	0.503	0.493	0.624	0.302

Read method is :read and eject, Single wave length is (405).

Table(7): Antibody levels of broiler chicken immunized with C. perfringens toxo A, bacterin A and mixed toxoid A & C (one dose of the vaccine)

Time of serum collection	The optical density (O.D) of serum of broiler			
	Immunized with bacterin A	Immunized with bacterin C	Immunized with bacterin AC	Nonimmunized control group.
Zero time	0.646	0.523	0.220	0.502
2 week post immunization	0.754	0.741	0.807	0.604
3 week post immunization	0.803	1.125	1.190	0.606
4 week post immunization	0.911	0.417	0.932	0.644