Efficacy of IgY immunoglobulin prepared in chicken egg yolk for the protection of chicken against necrotic enteritis

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A B S T R A C T

This study was designed to provide a rapid highly protective passive immunization of chickens against necrotic enteritis (NE). This aim was established by preparation of NE alpha toxin IgY in chicken egg yolk, such preparation was found to have specific anti NE alpha toxin titer 40 I.U by SNT and 0.237 optical density (OD) by ELISA. It was found that oral administration of 40, 20, 10 and 5 IU/ml of IgY / poult after experimental infection with *Clostridium perfringens* type A, resulted in protective rates of 96%, 88%, 80% and 60% respectively. Chickens' sera of passively immunized birds showed antibody titers of 1, 2 and 1.5 I U in the first, second and third days' post immunization respectively. It was concluded that IgY for NE alpha toxin type A could be used successfully to protect or even minimize the severity of the disease during possible outbreaks.

Keywords: NE, *Clostridium perfringens,* Alpha toxin, *IgY*

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1. INTRODUCTION

Poultry is one of the fastest growing segments of the agriculture sector. Poultry meat is an important source of animal protein. Most importantly, there's a demand for meat of high nutritional value and free from microbiological and chemical hazards. Enteric diseases are important concern to the poultry industry because of loss of productivity, increased mortality, and the associated contamination of poultry products for human consumption (Diraviyam et al., 2011). Necrotic enteritis (NE) is one of the most important enteric diseases caused by Gram – positive *Clostridium perfringens* type A strain and it has gained more attention in the broiler industry (Yanlong et al., 2015). *C. perfringens* is a commensal in the gut of vertebrates including poultry. Development of clinical NE appears to be at least partially dependent on the presence of predisposing factors such as prior damage to the mucosal surface of the intestine or a diet containing fishmeal or high levels of indigestible polysaccharides, as found in rye, wheat and barley (Crouch et al., 2010). Necrotic enteritis (NE) has been controlled by adding antibiotics in the feed of poultry, but removal of in-feed antibiotics can influence the effectiveness of preventive strategies for this disease (Yegani and Korver, 2007). There's concern that antibiotic resistance in bacteria may make the commonly used antibiotics less effective. So, oral immunotherapy (passive immunization) with specific antibodies is a strategy that has been actively pursued in laboratory and clinical studies for the last two decades. Feeding of egg yolk antibodies to neutralize specific pathogens, especially enteric microorganisms, are potential alternative to antibiotics by binding, immobilizing and consequently reducing or inhibiting the growth, replication, or colony forming abilities of these pathogens (Yegani and Korver, 2007).

The present work aims to provide rapid specific protection of chicks against *Clostridium perfringens* type A infection especially in emergency cases. Such aim was established through preparation of immunoglobulins against alpha toxin of *Clostridium perfringens* type A in chicken egg yolk which given orally in chicken to provide an acceptable protection rate against NE.

2. MATERIAL AND METHODS

2.1. Laying Hens:

Ten commercial laying white leghorn hens of twenty-six weeks old in good health were obtained from Koum Osheim farm Fayoum Egypt and used

for production of IgY against alpha toxin of *Clostridium perfringens*.

2.2. Poults:

Two hundred SPF poults (two weeks old) were used in assay to measure the efficacy of produced IgY.

2.3. Bacterial strain:

Clostridium perfringens type A locally isolated strain from chicken suffered from NE, this strain was isolated and identified in Anaerobic Bacterial Vaccine Research Department of VSVRI. It was used for production of NE vaccine. It was used for experimental infection of poults.

2.4. Necrotic enteritis (NE) Vaccine:

One bottle from the currently prepared NE vaccine in department of anaerobes was taken to inject layed egg hens for production of IgY.

2.5. Production of egg yolk IgY:

Twenty-six weeks old ten egg layer hens were injected with 0.5 ml Sub cutaneous of NE vaccine twice with two weeks apart and then every week started after two weeks from second injection and for five weeks. Blood samples and eggs from these hens were collected after one week from second vaccination and then every week for five successive weeks until 2 weeks after last injection, the time that antibody of NE decreased in sera and egg yolk. IgY was extracted from egg yolk according to Ikemori et al. (1997). The egg yolk was separated, pooled and kept at -20 ^{o} C.

2.6. Isolation, extraction and purification of IGY:

The isolation, extraction and purification of water-soluble fraction of IgY carried out as described by Akita and Nakai (1993).

2.7. Experimental design:

Two hundred poults were divided into eight groups (25 chicks /group) and treated as in table (1) briefly according to Roukaia Osman et al. (2008) Four groups of them (groups 3,4,5,6) were starved for about twenty hours, then fed on feed containing a 18 hours broth cultures of *C. perfringens* type A for five successive days (ratio of feed to broth was 1:15) till appearance of signs of disease and then these groups were fed on egg yolk IgY after 24 hours from appearance of disease symptoms. The other four groups, one group (group 2) of them received egg yolk IgY then after three days received with *C. perfringens* type A culture. the second group (group 1) received IgY only. The third group (group 7) received *C. perfringens* type A culture, and the last group (group 8) left without infection or treatment as control group.

2.8. Screening of chicken anti - clostridia alpha toxin sera and IgY:

Detection and evaluation of antibody titers against clostridia alpha toxin in chicken sera and egg yolk samples were carried out using serum neutralization test (SNT) according to Gadalla et al. (1997), and enzyme linked immunosorbent assay (ELISA) according to Matter et al. (2002).

Table (1): Experimental Design

Groups	Route of administration	Scheme of treatment
1	Orally	Chicks received the prepared IgY only
2		Received 40 IU/ml of the prepared IgY then experimentally infected with *Clostridium perfringens* type A culture after 3 days.
3		Experimentally infected with *Clostridium perfringens* type A culture till appearance of disease signs then received 40 IU/ml of the prepared IgY
4		Experimentally infected with *Clostridium perfringens* type A culture till appearance of disease signs then received 20 IU/ml of the prepared IgY
5		Experimentally infected with *Clostridium perfringens* type A culture till appearance of disease signs then received 10 IU /ml of the prepared IgY
6		Experimentally infected with *Clostridium perfringens* type A culture till appearance of disease signs then received 5 IU /ml of the prepared IgY
7		Experimentally infected with *Clostridium perfringens* type A culture (control positive)
8		It was kept as not-infected and not received IgY (control negative)

3. RESULTS

As shown in table (2). On the fourth week post hen immunization, it was found that the obtained IgY had specific *Clostridium perfringens* type A antibody titers of 40 IU/ml and 0.237 O.D. by SNT & ELISA respectively. The sera of hens used for production of IgY had a titer of 10 IU/ ml and 1.367 O.D. by SNT & ELISA respectively. As shown in table (3) chicks received culture of *Clostridium perfringens* type A and then 24 hours after appearance of signs administrated IgY in doses 40, 20, 10 and 5 IU/ml IgY per chick showed protection against *Clostridium perfringens* type A with percentage 96%, 88% ,80% and 60% respectively.

In group (2) which treated with culture of *Clostridium perfringens* type A after 3 days of receiving 40IU/ml of the prepared IgY resulted in no protection against NE disease. In group (1) the group that received IgY only and not infected with *Clostridium perfringens* type A exhibited specific antibodies with SNT titers of 1, 2,1.5 and 1 IU/ml by the 1st, 2nd, 3rd, 4th day post passive immunization respectively.

Table (2): Mean of serum antibodies and IgY in immunized hens against alpha toxin of *Clostridium perfringens* type A:

Week post immunization	Mean NE antibody measurements			
	SNT I.U/ml		ELISA O.D.	
	Serum	Yolk	Serum	Yolk
Pre-immunization	0	0	0.01	0.02
Zero day	0	0.02	0	0.01
First week	2.5	10	0.265	0.066
Second week	5	15	0.324	0.097
Third week	8	25	0.572	0.138
Fourth week	10	40	1.367	0.237

At fifth and sixth weeks mean NE antibody titer of IgY and sera decreased.

N.B.: ELISA OD of serum above 0.332 is considered positive. ELISA OD of IgY above 0.1 is considered positive. The minimum protective level for *Clostridium perfringens* type A is 0.5:4 I.U/ ml.

Table (3): Effect of different doses of anti-clostridia alpha toxin IgY after experimental infection with *c. perfringens* type A culture

Days post treated with IgY	Groups No.	Dosage of IgY(IU/ml)	No. of treated poults	No. of poults showing symptoms or dead	P/M	Protection %
During three days	3	40IU/ml	25	1	Showing hemorrhagic enteritis	96%
	4	20IU/ml	25	3		88%
	5	10IU/ml	25	5		80%
	6	5IU/ml	25	10		60%

4. DISCUSSION

Necrotic enteritis has been reported from most areas of the world where poultry is produced. The main cause of the disease is *Clostridium perfringens* type A. Toxin produced by this bacterium are responsible for intestinal mucosal necrosis, the characteristic lesion of Necrotic Enteritis. So, the present study focused to develop egg yolk antibody to control the morbidity and mortality in affected chicks by the *Clostridium perfringens* type A instead of treating the infected

birds using antibiotics in a parallel way with Diraviyam et al. (2011) where rapid protection can be achieved by passive transfer of hyper immune serum (Blancou, 2002).

As shown in table (2), immunization of chickens with necrotic enteritis vaccine induced high antibody titer in chicken sera and the egg yolk. The antibody titer started to increase from the first week post immunization with NE vaccine and continued to increase after the second immunization reached the highest protective level at the fourth week post immunization (40 IU/ml) and decreased at fifth and sixth weeks. The sera of hens used for preparation of IgY had titers of 10IU/ml and 1.367 OD by SNT and ELISA respectively. In this respect, Ulmer- Franco (2012) chicken antibodies are produced by immunization of laying hens and subsequently purification of IgY from the egg yolk. Also, maternal antibodies are transferred from serum to egg yolk. Concentration of IgY in the yolk is higher than that in the chicken serum.

These results showed that, the antibody titers in the chicken sera came in parallel with those obtained in their egg yolk.

On the other side, young chicks were infected experimentally with culture of *Clostridium perfringens* type A for 5 successive days and after appearance of disease signs by 24 hours, they received different doses of anti-clostridia alpha toxin IgY (40, 20, 10 and 5 IU/ml/bird). provided protection rates of 96% and 88%, 80% and 60% respectively to chicks against experimental infection. These findings came to be confirmed by those of Diraviyam et al. (2011) who concluded that purified chicken antibodies can be used for passive immunization to protect young chickens from enteric infection. Also, similar conclusion was reported by Tamilzarasan et al. (2009). Control non-immunizes chicks showed typical NE signs represented by depression, decreased appetite, less movement and death as what recorded by Wages and Opengart (2003) with post mortem findings of hemorrhagic enteritis (Olkowski et al., 2006).

Chicks immunized with anti-clostridia alpha toxin IgY only exhibited antibody titer of 1 I.U/ml then reached to the peak at the second day (2 IU/ml) then begin to decreased gradually at third and fourth days reached to be (1.5 and 1 IU/ml) but remain protective to the fourth day, as the protective level of *C. perfringens* type A toxins in chicken is (0.5 – 4 IU/ml) (central lab quality control). This finding confirms the usefulness of the prepared anti- *C. perfringens* type A IgY in chicken egg yolk to be used for passive immunization of chicks against the disease infection. In this respect Gamal et al. (2015) found that Chicken egg yolk IgY induced a short life span immunity (4 days) in passively immunized hosts, but it can be used simultaneously or with conventional emergency vaccination to protect susceptible hosts during disease outbreaks and incubation period of infection. Yegani and Korver (2007) concluded that egg yolk antibodies may act against enteric pathogen by binding, immobilizing and consequently reducing or inhibiting the growth, replication, or colony forming abilities of these pathogens.

5. CONCLUSION

It could be concluded that, the passive protection of chicken before infection occur has no significance, but it could be used as an emergency during an outbreak or even after appearance of signs of disease within three days to protect chickens during the incubation period or to overcome the undesirable infection effects.

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