DETERMINATION OF THE RELATIONSHIP BETWEEN EGG DROP SYNDROME (EDS) IMMUNE STATUS IN VACCINATED HENS AND THEIR EGG QUALITY

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Abstract

The present work was carried out in a trial to overcome the possible obstacles which may face the evaluation of egg drop syndrome (EDS) vaccine through the application of the challenge test using the virulent virus. It spots the light on the relationship between the levels of EDS antibodies in vaccinated hens and the quality and quantity of laid eggs from these hens. Live attenuated chicken embryo fibroblast cell culture (CEF) and inactivated duck egg EDS vaccines were used to such purpose where they were used to vaccinate 2 groups of hens at 4 and 16 weeks of age. The induced antibodies in vaccinated hens were followed up using hemagglutination inhibition test (HI) and serum neutralization test (SNT) revealing that all vaccinated hens exhibited high levels of specific EDS antibodies confirming the production of good quality and quantity of eggs.

INTRODUCTION

Egg drop syndrome virus (EDS) is an adenovirus (Baxendale, 1978) affecting egg production and quality in laying hens through virus replication in the uterus and lower regions of the oviduct. The virus is transmitted vertically, and the appearance of the disease around the peak of egg production may be due to reactivation of latent virus (Mcferran, 1979). Other signs of EDS infection may be recorded as inappetence and transient diarrhoea which is probably due to the exudates from the oviduct (Smyth *et al*., 1988).

The syndrome was recorded in Egypt for the first time by Khafagy and Hamouda (1991) through the detection of HI antibodies against EDS-76 in commercial chicken layers, while, the causing agent was isolated by Ahmed (1995).

It was noticed that both normal and abnormal shelled eggs produced during the period of virus replication in the pouch shell gland contained virus on the exterior and anterior leading to contamination of egg trays (Cook and Darbyshire, 1980) and the presence of the virus in the feces arises from contamination by oviduct exudates (Smyth *et al*., 1988).

When vertical or lateral transmission of EDSV occurs, the flocks should be protected by vaccination in growing period. Birds would be vaccinated between 14 and

16 weeks of age, exhibiting immunity lasts at least one year (Solyom *et al.*, 1982). It was concluded that the inactivated vaccine is effective in the control of EDS-76 infection.

It was established that, challenge of vaccinated chickens against the virulent agent of the used vaccine is an essential step in the quality control of the tested vaccine, but, this step may lead to spread infection to other flocks under unrestricted hygienic measures or even with the carelessness of workers, in addition to the high cost of such test.

The present work tries to determine the relationship between the EDS antibody titer in vaccinated hens and the quality of their eggs to avoid any possible risks.

MATERIALS AND METHODS

Vaccines

Lyophilized EDS-76 live attenuated vaccine prepared on CEF cells was prepared locally in Veterinary Serum and Vaccine Research Institute with a titer of 12 $\log_{10}$ $TCID_{50}$ /ml (Nadia and Abo zaid 2010).

Inactivated oil emulsion EDS-76 vaccine prepared on duck eggs was locally prepared in the same institute.

Attenuated EDS76 virus: EDS-76 virus adapted on chicken embryo fibroblast (CEF) cell culture (Nancy *et al.,* 2003) was used for serum neutralization test.

Chicken embryo fibroblast cell culture (CEF): Primary CEF cell culture was prepared according to Singh *et al* . (1967) and used in serum neutralization test for estimation of EDS serum neutralizing antibodies.

Three hundreds one day old Hubbard chicks were obtained from the United Company for Poultry Production. These chicks were divided into 3 groups each of 100 birds reared under hygienic measures where the first group was vaccinated with 2 doses of the live attenuated vaccine prepared on CEF cell cultures at the 4^{th} and 16^{th} week of age, respectively. The second group was vaccinated with the duck egg inactivated vaccine at the same periods, while, the third group was kept as non-vaccinated control.

Each bird in the groups vaccinated with the live EDS 76 vaccine was installed intra-ocular with a dose containing at least 10^6TCID_{50}, whereas chickens vaccinated with the inactivated EDS76 vaccines received a dose of 0.5ml/ bird inoculated I/M. Twenty random serum samples were obtained from each chicken

group weekly up to the first 4 weeks, then every 4 weeks (monthly) up to 16 weeks of age (the time of the second vaccination) ,and then, weekly up to 20 weeks of age (4 weeks post the second vaccination), and then, monthly up to 8 months of age (32 weeks post- the last vaccination).

Serum neutralization test (SNT): It was performed as described by Roositer *et al* . (1985) and the antibody titer was calculated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of EDS virus according to Singh *et al.* (1967).

Haemagglutination inhibition test (HI): It was carried out according to Anon (1971).

Egg curve: It was drawn to evaluate the quantity and quality of eggs produced by vaccinated chickens.

RESULTS

Table 1. Mean titers of serum neutralizing EDS antibodies in vaccinated chickens.

Used vaccine	Mean EDS serum neutralizing antibody titer												
	↑ 4 WA * (1st Vac.) ** ↓	5 WA	6 WA	7 WA	8 WA	↑ 16 WA (2nd Vac.) *** ↓	17 WA	18 WA	19 WA	20 WA	24 WA	28 WA	32 WA
Live CEF		8	16	24	32		40	64	128	128	128	128	128
Inac. duck egg		4	8	18	30		40	64	64	128	128	128	128
C		0	0	0	0		0	0	0	0	0	0	0

*WA= week of age

**1st Vac. = first vaccination ***2nd Vac. = second vaccination.

Table 2. Mean HI titers of EDS antibodies in vaccinated chickens.

Used vaccine	Mean HI titers of EDS serum antibodies (log2/ml)												
	↑ 4 WA * (1st Vac.) ** ↓	5 WA	6 WA	7 WA	8 WA	↑ 16 WA (2nd Vac.) *** ↓	17 WA	18 WA	19 WA	20 WA	24 WA	28 WA	32 WA
Live CEF		4	8	11	11		12	12	13	11	12	12	12
Inac. duck egg		2	4	8	9		11	12	13	12	11	13	12
C		0	0	0	0		0	0	0	0	0	0	0

*WA= week of age

**1st Vac.= first vaccination ***2nd Vac.= second vaccination

Table 3. Mean quantities of egg production with good quality starting from 16 weeks of age.

Used vaccine	Number of hens	Mean quantities of egg production/ weeks of age				
		16WA*	20WA	24WA	28WA	32WA
Live CEF	100	300	320	310	295	300
Inactivated duck egg	100	290	285	315	300	275
Control	100	270	227	299	307	298

*WA=week of age

DISCUSSION

This work has investigated the correlation between the EDS immune status in vaccinated hens and the quality and quantity of produced eggs avoiding the application of challenge test to evaluate the vaccine potency.

Maternal antibodies against EDS were screened in the experimental chickens at 4 weeks of age before vaccination, and it was found that they were free from such antibodies. All vaccinated chickens did not show any abnormal signs allover the experimental period confirming vaccine safety. SNT and HI results revealed that vaccination of chickens at 4 weeks and revaccinated at 16 weeks of age before starting of the season of egg production induced high levels of antibodies and egg production. The results of serum neutralization test (SNT) as shown in Table 1 showed the maximum value (128), and Table 2 showed the highest HI titer (13log2) by the 3rd week post- the 2nd vaccination and still high up to 32 weeks of age (16weeks post-boostering), coming in agreement with what reported by Rhee *et al.* (1987) and Nadia (2004&2010). These determined titers appear to be sufficient to protect hens against virus infection as stated before by Baxendale (1978) and Khodeir and Amina (1999). In addition, Khalaf *et al* . (1982) and Nadia (2010) recorded high protective titers of EDS antibodies between the 2nd and 5th week post-the 2nd vaccination.

Also, vaccination programs in young age (4weeks) followed by another dose before the period of egg production was suggested and confirmed by Kaur *et al.* (1997) and Kozlina *et al* . (1990) providing high immunity to chicks .

On investigation of the mean quantity and quality of the egg production (Table 3) of vaccinated chicks, it was noticed that there was no abnormality in the quality of laid eggs with good level of production in comparison to the healthy unvaccinated well managed and fed hens indicating that the prepared vaccines are effective to withstand EDS76 virus infection and protect the chicks against both production drop and poor quality of eggs as recommended by Cook and DarbyShire (1980). The irregular increase and decrease in the quantity of egg production in vaccinated and unvaccinated groups appears to be within the normal status of chicks, in agreement with what mentioned by McFerran (1979), Friederichs *et al*. (1987) .

So, the immune status of EDS in hens can reflect the quality and quantity of egg production and vis-a -vis.

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تحديد العلاقة بين الحالة المناعية لظاهرة تدنى البيض فى الدجاج المحصن ونوعية البيض

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تم إجراء هذه الدراسة كمحاولة للتغلب على تطبيق اختبار التحدى عند تقييم فاعلية لقاح ظاهرة تدنى البيض باستخدام الفيروس الضارى الأمر الذى يمثل جانبا من خطورة إنتشار الفيروس تحت أيّ ظروف وتوفيرا لتكلفة اختبار التحدى.

تم تحصين مجموعتين من الدجاج بلقاحين أحدهما نسيجى حى مستضعف والآخر مثبط محضر فى أجنة بيض البط بتقديم جرعتين عند عمر 4 و16 أسبوع ثم تم تتبع مستويات الأجسام المناعية المتكونة باختبارى منع التلزن الدموى والمصل المتعادل حيث أوضحت نتائج الاختبارين أن الطيور المحصنة تكتسب مستويات مناعية جيدة تكفى لحماية الدجاج ضد الفيروس الضارى مع إنتاج بيض ذي جودة عالية وبقيم تقارب مثيلاتها فى الطيور السليمة التي تربي تحت ظروف صحية جيدة وتتناول علائق متورازنة. وعلى ذلك يمكن القول بأن الحالة المناعية للدجاج ضد ظاهرة تدنى البيض تدل على نوعية البيض الناتج من حيث الكم والكيف.