VACCINATION PROGRAMME FOR EGG DROP SYNDROME (EDS) USING LOCAL PREPARED VACCINES

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Abstract

The present work deals with 9 different trials as vaccination programmes against egg drop syndrome (EDS) using three locally prepared vaccine formulae including cell culture, live attenuated and inactivated vaccines (prepared on chicken embryo fibroblasts), and inactivated duck egg vaccine. The applied programmes include vaccination of young chicks at 4 weeks of age and older ones at 19 weeks of age prior to the start of egg production. Also, these programmes include vaccination of such birds with one and two doses. Evaluation of the applied vaccination programmes was depending on the estimation of specific induced EDS antibodies using serum neutralization test (SNT) and Haemagglutination test (HI), in addition to the egg production curve.

All vaccinated chickens were still healthy allover the experimental period showing no abnormal signs ,confirming safety of the vaccine, and were able to withstand the challenge virus , while, unvaccinated birds did not withstand it.

The obtained results revealed that the best vaccination programme is that which included vaccination of chickens at 4 weeks and then revaccinated at 19 weeks of age before starting of the season of egg production (programmes 4,5&6 "VP4, VP5 & VP6) and resulted in the highest levels of antibodies and egg production.

INTRODUCTION

Egg drop syndrome EDS is a viral disease of layers caused by an adenovirus with an economic importance characterized by depressed egg production, laying of soft – shelled or shell less eggs and failure to reach peak production (Van Eck *et al.,* 1976, McFerran *et al.,* 1978).

The effect of EDS virus on egg production and quality in laying hens has been shown experimentally to be done by virus replication in the uterus and lower regions of the oviduct (Higashihera *et al.*, 1987).

Chickens of all ages are susceptible to EDS virus infection where it is transmitted vertically. However, the appearance of the disease at peak egg production may be due to reactivation of latent virus (Higashihara *et al.*, 1987, Mcferran *et al.,* 1978).

Affected birds remain otherwise healthy although inappetence and dullness have been described in some affected flocks. Transient diarrhea is probably due to the

exudates from the oviduct, (Smyth *et al.*, 1988). EDS virus does not cause clinical disease in growing chickens in all fields. Oral infection of susceptible day-old chicks resulted in increasing mortality in the first week of life (Smyth *et al.*, 1988, Cook and Darbyshire, 1981).

In Egypt, Khafagy and Hamouda (1991) reported on the first record for prevalence of HI antibodies against EDS-76 in commercial chicken layers, and Ahmed (1995) succeeded, for the first time, to isolate the virus from chicken farms.

It is now possible to divide EDS outbreaks into three types, classic form of the disease where primary breeders (laying Hens)were infected, and the main method of spread was vertically through the embryonated egg (Mc Ferran *et al.*, 1978). Lateral spread of the virus was very efficient. In many cases chicks infected in ova did not excrete greater than 50% egg production and at this stage, the virus was reactivated and excreted resulting in rapid spread due to multiple foci of infection (Baxendale *et al.*, 1980).

The endemic form was often associated with a common egg packing stating. Both normal and abnormal shelled eggs produced during the period of virus growth in the pouch shell gland contained the virus on the exterior and anterior .This led to contamination of egg trays; dropping also contained the virus (Cook and Darbyshire, 1980).

In adult birds, presence of the virus in the feces arises from contamination by oviduct exudates (Smyth *et al.*, 1988). Direct spread between birds, could occur during transported needles used for vaccination (Van Eck *et al.*, 1976 and Cook and Darbyshire, 1981) who represented a third type of disease outbreak. Spread of EDS virus from domestic or wild ducks , geese are another wild birds to hens through drinking water contaminated by dropping. This is very important due to cases tending to be sporadic, but there is always the danger of an infected flock becoming the focus for endemic infection. When vertical or lateral transmission of EDSV occurs, the flocks can be protected by vaccination in the growing period. The infected egg is the most dangerous source of the virus. An oil adjuvant inactivated vaccine is widely used and provides a good protection against clinical EDS. The birds were vaccinated between 14 and 16 weeks of age. Vaccine immunity lasts at least one year. It was concluded that the inactivated vaccine is effective in the control of EDS-76 infection ,and protects the fowl against both drops in egg production and the production of poor quality eggs, in addition to good protection against challenge (Baxendale *et al.*, 1980).

So, the aim of this work is the designation of a suitable and effective programme for chicken vaccination against EDS-76 using locally prepared vaccines.

MATERIALS AND METHODS

1. Vaccines

1.1- 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 2004).**

1.2- EDS76 inactivated vaccines:

Two inactivated oil emulsion EDS-76 vaccines prepared on CEF cells and on duck eggs were locally prepared in the same institute.

2-Attenuated EDS76 virus

EDS-76 virus adapted on chicken embryo fibroblast (CEF) cell culture (Nadia 2004 and Nancy *et al.*, 2003) was used for serum neutralization test.

3-Virulent EDS76 virus

The virulent virus was supplied by the same institute with a titer of $8log_{10}$ EID_{50}/ml and used for challenge of experimental birds.

4-Chickens

One thousand – six hundred one day old Hubbard chicks were obtained from the United Company for Poultry Production.

5-Experimental design

The experimental chicks were divided into 10 groups each of which comprised 160 birds being managed as follows:

Group-1 represents the 1st vaccination programme **(VP1)** which was vaccinated at 4 weeks of age with one dose of the live attenuated vaccine prepared on CEF cell cultures.

Group-2 represents the 2nd vaccination programme **(VP2)**which was vaccinated at 4 weeks of age with one dose of the inactivated vaccine prepared on CEF cell cultures.

Group-3 was subjected to the 3rd vaccination programme **(VP3)** where the chicks were vaccinated at 4 weeks of age with one dose of the inactivated vaccine prepared in duck eggs.

Group-4 received the 4th vaccination programme **(VP4)** where these birds were vaccinated at 4 weeks of age with the inactivated CEF vaccine and revaccinated with the same vaccine on the 19th week of age.

Group-5 was subjected to the 5th vaccination programme **(VP5)** where the chicks were vaccinated at 4 weeks of age with the inactivated vaccine prepared in duck eggs and revaccinated with the same vaccine at 19 weeks of age.

Group-6 represented the 6th programme **(VP6)** where the chicks were vaccinated at 4 weeks of age with the live attenuated CEF vaccine, then ,with the inactivated CEF vaccine at 19 weeks of age.

Group-7 subjected to the 7th programme **(VP7)** where the chicks were vaccinated with one dose of the CEF live attenuate vaccine at 19 weeks of age.

Group-8 represented the 8th programme **(VP8)** where the chicks were vaccinated with one dose of the inactivated CEF vaccine at 19 weeks of age.

Group-9 Chicks were vaccinated with the duck egg inactivated vaccine at 19 weeks of age representing the 9th programme **(VP9).**

Group -10 Chicks were kept as non -vaccinated control chickens **(VP0).**

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 chicken vaccinated with the inactivated EDS76 vaccines received a dose of 0.5ml/ bird inoculated I/M.

Ten random blood samples were obtained from each chicken group weekly for the first three months , and then, every 2 weeks up to 18 weeks post-vaccination.

Serum samples were separated and tested for estimation of neutralizing antibodies against EDS76 virus using serum neutralizing test, and also, to detect Haemagglutination activity by using Haemagglutination inhibition test (HI).

6-Challenge test

It was performed as described by Friederichs *et al.*, (1987).

7-Serum neutralization test (SNT)

It was performed as described by Rossiter *et al.*, (1985).

8-Haemagglutination inhibition test (HI)

It was carried out according to Anon (1971).

9- Egg curve

It was drawn to evaluate the quantity and quality of eggs produced by different chicken groups.

RESULTS

Table 1. Mean EDS neutralizing antibody titers in vaccinated chickens with one dose of different prepared EDS vaccines at 4 weeks of age.

Programme number	Used vaccines	Mean EDS neutralizing antibody titer (log2/ml) / Weeks post- vaccination																														
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
VP1	Live attenuated EDS on CEF	10	11	11	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	11	11	11	11	11	10	10	10	10	9	9	9
VP2	Inactivated EDS 76 on CEF	5	5	8	10	12	12	12	12	12	12	12	12	12	12	11	10	10	10	9	9	8	8	8	7	7	7	7	6	6	6
VP3	Inactivated EDS 76 on Duck egg	4	4	8	9	11	12	12	12	12	12	12	12	12	12	11	11	11	10	10	9	9	8	8	8	7	7	7	6	6	6
0	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. Mean EDS neutralizing antibody titers in chicks vaccinated with 2 doses of inactivated vaccines.

Programme number	Used vaccines	log2 antibody titer /Weeks after vaccination														Log2 antibody titer/Weeks post- boostring																							
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	29	26	28	30	32	34	36	38	40	42	44	46	48
4	Inactivated CEF	5	5	8	10	12	12	12	12	12	12	12	12	12	13	14	14	15	15	15	15	15	14	14	14	13	13	14	13	12	12
5	Inactivated on duck egg	4	4	8	9	11	12	12	12	12	12	12	12	12	13	14	15	15	15	15	15	14	14	14	14	14	14	13	14	14	14
6	Live attenuated CEF then inactivated CEF	10	11	11	12	12	12	12	12	12	12	12	12	12	13	14	14	14	15	15	15	14	14	13	12	12	11	11	11	11	10
0	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Mean EDS neutralizing antibody titers (log 2) in sera of chick vaccinated with different vaccines at 19 weeks of age.

Programme number	Used vaccine	Weeks after vaccination																
		1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33
VP7	Live attenuated CEF vaccine	9	12	14	14	15	15	15	14	14	13	12	12	11	11	11	11	10
VP8	Inactivated (CEF)	7	8	10	12	13	13	14	15	15	14	14	13	12	12	12	12	11
VP9	Inactivated on duck egg	8	9	11	12	13	15	15	14	13	13	13	13	12	12	12	12	12
0	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Mean EDS HI antibody titers in vaccinated chickens with one dose of different prepared EDS vaccines at 4 weeks of age.

Programme number	Used vaccines	Mean EDS neutralizing antibody titer (log2/ml) / Weeks post- vaccination																													
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
VP1	Live attenuated EDS on CEF	6	6	8	8	9	9	10	8	8	7	7	7	7	7	6	6	5	5	5	5	5	4	4	4	4	3	3	2	2	6
VP2	Inactivated EDS 76 on CEF	5	5	7	9	11	8	8	7	7	7	6	6	6	6	6	5	5	5	5	5	4	4	4	4	3	3	3	3	3	5
VP3	Inactivated EDS 76 on Duck egg	9	10	9	8	8	6	7	7	7	7	7	7	7	7	6	6	5	5	5	5	5	4	4	4	4	3	3	3	3	9
0	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5. Mean EDS HI antibody titers in chicks vaccinated with 2 doses of inactivated vaccines.

Programme number	Used vaccines	log2 antibody titer /Weeks after vaccination													Log2 antibody titer/Weeks post- boostring															
		1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	29	26	28	30	32	34	36	38	40	42	44	46	48
4	Inactivated CEF	5	5	7	9	11	8	8	7	7	7	6	6	6	7	8	9	9	9	9	9	8	8	8	7	7	7	7	6	6	5
5	Inactivated on duck egg	9	10	9	8	8	6	7	7	7	7	7	7	7	8	8	9	9	9	9	9	8	8	8	7	7	7	6	6	6	9
6	Live attenuated CEF then inactivated CEF	5	6	6	5	9	6	10	7	7	8	7	7	7	9	9	9	10	10	10	12	12	12	11	11	11	9	9	8	7	7
0	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 6. Mean EDS HI antibody titers (log 2) in sera of chick vaccinated with different vaccines at 19 weeks of age.

Programme number	Used vaccine	Weeks after vaccination																
		1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33
VP7	Live attenuated CEF vaccine	5	6	9	9	9	9	9	8	8	8	8	6	6	6	5	4	4
VP8	Inactivated (CEF)	5	5	6	7	8	9	9	9	8	8	8	6	6	6	5	4	4
VP9	Inactivated on duck egg	6	7	8	8	9	9	9	8	8	7	7	6	6	6	5	5	4
0	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 7. Mean quantity of egg production of vaccinated chicks with different prepared EDS-76 vaccines.

Chicken group	Used vaccine	Number of chicks	Number of eggs per week														Ratio percentage
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	Live on (C.E.F)	20	14	40	25	4	10	30	25	20	10	0	5	20	10	10	11.65 %
2	Inactivated (C.E.F)	20	25	35	15	10	20	30	15	20	20	15	10	25	25	15	13%
3	Inactivated on duck egg	20	30	35	25	5	21	26	18	21	21	15	12	26	27	15	14%
4	Control	20	2	4	5	5	7	5	7	7	7	4	6	7	5	5	4 %

Table 8. Mean quantity of egg production of vaccinated chicks with 2 doses of the inactivated locally prepared EDS vaccines.

Chicken group	Type of vaccine used	Number of chicks	Number of egg per week														Ratio percentage of egg production
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	Inactivated CEF then inactivated CEF	20	5	15	12	14	18	0	15	30	40	90	50	60	60	20	19 %
2	Inactivated on duck egg then inactivated duck egg	20	10	16	6	0	26	26	18	26	46	18	16	40	50	30	16.4 %
3	Control	20	0	0	5	5	8	8	10	10	15	15	12	10	5	5	4 %

Table 9. Mean quantity of egg production of vaccinated chicks with the locally prepared live attenuated vaccine and boostered with inactivated locally prepared vaccines .

Chicken group	Type of vaccine used	Number of chicks	Number of egg per week														Ratio of egg production percentage
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	Live on (CEF) then inactivated CEF	20	25	20	25	20	23	25	20	30	35	28	20	15	10	5	.15 %
2	Control	20	0	0	5	5	8	8	10	10	15	15	12	10	5	5	4 %

DISCUSSION

The present study deals with suitable and effective programme for vaccination of chickens against EDS-76 virus using different locally prepared vaccines.

Maternal antibodies against EDS were investigated in chickens at 4 weeks of age before the application of experimental vaccination programmes ,and it was found to be of no electotrial value.

All vaccinated chickens were still healthy allover the experimental period showing no abnormal signs confirming safety of the vaccine, and were able to withstand the challenge virus, while unvaccinated birds did not withstand it.

The obtained results revealed that the best vaccination programme is that which included vaccination of chickens at 4 weeks, and then, revaccinated at 19 weeks of age before starting of the season of egg production (programmes 4,5&6 "VP4, VP5 & VP6) and resulted in the highest levels of antibodies and egg production. The results of serum neutralization test (SNT) as shown in Tables (1-3) where different groups of chicks vaccinated at one 4 weeks of age, revealed that the highest neutralizing antibody titers in all vaccinated groups with the maximum value (12 log 2) were recorded at the 4th weak till 24th week post-vaccination coming in agreement with what reported by Lee,A.M.T. and Hopkins (1982) and Rhee *et al* .,(1987).

In chicks vaccinated at 19 weeks of age(VP7, VP8 and VP9) with one dose of the locally prepared inactivated and live attenuated (EDS_{76}) vaccines, it was noticed that there was gradual increase in mean neutralizing antibody titer from one week post-vaccination and reached to high level in weeks 26-28 after vaccination with (15 log2). Also, there was an increase in mean neutralizing antibody titers in old age than in young age of chicks. That is the thing which could be attributed to the affinity of (EDS_{76}) virus to reproduce and the maturity of the immune system.

On comparison between the different programmes it appears clearly that the mean neutralizing antibody titers in programmes (VP4, VP5 &VP6)) induced the highest immune response to all vaccinated groups with inactivated or live attenuated vaccines recording their maximum from the week 16th till the 48th weeks reaching (13-14-15 log 2) ,and then, decreased by the 48th week post-vaccination (14-13-12 log 2) providing highly protection to vaccinated chicks in the period of egg production. Such vaccination programmes in young age (4weeks) followed by another dose before the period of egg production was suggested and confirmed by Kaur *et al.* (1997).

In programmes (VP7, VP8 and VP9)) where chicks were vaccinated at old age before the time of egg production, it was noticed that maximum neutralizing antibody titers appeared from the 2nd week (13-12 log 2) till the 48th week of age (12 log 2)

meaning high neutralizing antibodies during the season of egg production (12-13-14-15 log 2) and providing high immunity to chicks. These are in agreement with Khalaf (1981).

The results of HI antibodies as shown in Tables (4,5&6) showed gradual increase in HI titers beginning from the second week post-vaccination till the 28th week ,then, gradually decreased till the 48th week with maximum titers in week 4,5,6 (9-8 Log 2). Similar results were obtained by Bouquet *et al.* (1980). Usually, the results of SNT and HI were nearly parallel to each other. In addition, the HI findings appear to be supported by Prommuang *et al.* (1999).

On investigation of the mean quantity and quality of the egg production of vaccinated chicks with different prepared (EDS76) virus vaccines (Tables 7,8&9), it was noticed that there was an increase in the quantity of egg production with good quality indicating that the prepared vaccines are effective to withstand EDS76 virus infection, and capable to protect the chicks against both production drop and poor quality of eggs as recommended by Baxendale *et al.* (1980) and Cook and Darbyshire (1981) who stated that oil adjuvant inactivated vaccine is widely used in birds between 14 and 16 weeks of age inducing good protection against clinical disease and reducing amount of virus excreted.

Also, these tables revealed irregular increase and decrease in the quantity of egg production in all vaccinated groups ;a fact which appears to be within the normal status of chicks. This is in agreement with what mentioned by Mc Ferran (1979), Friederichs *et al*. (1987) .

So, it could be concluded that programmes including chickens vaccination with inactivated oil emulsion vaccine or with live attenuated vaccine at young age and before the season of egg production ,and vaccination of chicks at old age [before season of egg production] with inactivated oil emulsion vaccine or live attenuated vaccines are the programmes of choice.

REFERENCES

1. Ahmed, M.H.H. 1995.Viruses associated with dropping production. Thesis Ph.D, Fac. Vet. Med, Cairo University
2. Anon, M. 1971. Method for examination of poultry biologivs and identifying and quantifying avain pathogens.National Academy of science, Washington, D.S. U.S.A
3. Baxendale, W.D., R. Hein Lutticken and Mcpherson. 1980.The results of field trials conducted with uninactivated vaccine against the egg drop syndrome 76 (EDS) 76.Avian pathol.9 (1): 77-91.
4. Baxendale, W. 1978. Egg drop syndrome 76.vet. Rec. 12:285-286.
5. Bouquet, J.F., B.Devaux, D.Gaudry, Y.Moreau. 1980.Vaccination against egg drop syndrome (EDS) & Newcastle disease with bivalent inactivated vaccine in oily adjuvant.Proceedings of 29th Western Poultry Disease Conference, Acapulco, Mexico, 22-25, April: (1980).
6. Cook J.K.A. and J.H. Darbyshire. 1981. Longitudinal studies on the egg drop syndrome 1976 (EDS76) in the fowl following experimental infection at 1-day old.Avian pathol. 10:449-459.
7. Friederichs, M. Siegmann, O. Heffels Redmann, U. Kaleta, E.F. and M. Neumann. 1987. Pathogensis of egg drop syndrome 76 after experimental infection of day old chicks and laying hens.Berliner and Munchen Tierarztliche Wochenschrift, 100(2): 41-47.
8. Higashihara, M., M.M. Hiruma, I. Houdatsu, S. Takai, and M. Matumoto. 1987. Experimental infection of laying chickens with egg drop syndrome 1976 virus.Avian Dis.,31: 193-196.
9. Kaur,A., M.S. Obsevoi and Singh Amarjit . 1997. Neutralizing antibody and challenge response to live and inactivated avian adenovirus –I in broilers. Tropic. Anim. Hlth. Prod., 29(3) : 141-146.
10. Khafagy, A.K. and M.S.M. Hamouda. 1991.Studies on egg drop syndrome (1976). Existance of antibodies in Egyptian commercial chicken layers. Vet. Med. J., Giza, 39(2):287-292.
11. Khalaf, S.E. 1981.Field and laboratory experiments on immunizing of hens against egg drop syndrome 1976(EDS) 76. Inaugural Dissertation, Tierarztiliche Hochschule Hannover, 1981: PP.89
12. Lee,A.M.T., Hopkins. 1982. Development of a potency test for inactivated egg drop syndrome-76 vaccines. Development in Biological Standardization ,91: 65-74.

13. Mcferran, J.B., R.M. Mccrackcen, E.R. Mckillop, M.S. Mcmulty and D.S.Collins. 1978. Studis on a depressed egg production syndrome in Northern Ireland. Avian Pathol .7: 34-47.
14. Nadia, M.I. 2004.Preparation and evaluation of attenuated and inactivated egg drop syndrome virus vaccines adapted on chicken kidney cell culture. Egypt. Vet. Poul., Assoc., 6th Sci. Conf: PP. 360- 366.
15. Nancy, B. Rofail, Nadia M. Ibrahim Hala M. El- Makaky and Fekria A. El-Bordiny 2003. Trials for propagation of egg drop syndrome EDS -76 virus on different tissue culture types. Kafr- El- Sheikh Vet. Med. J., (1): 697-707.
16. Prommuang, P., C. Antarasena and P. Prommuang. 1999. Serological studies on egg drop syndrome virus infection in laying chicken.J. Thai. Vet. Med. Assoc.,50(1/2):53-59.
17. Rhea, Y.O, J.H. Kin and S. Namgoong. 1987. Immunogenicity of ND-EDS 76-IBD Combined oil adjuvnmat vaccine Research reports of this Rural Development Administration.Live stock and veterinary, 29(1): 209-212.
18. Rossiter, P.B., D.M. Tessett and W.P. Taylor. 1985. Microneutralization system for use with different strains of peste des petits ruminants virus and Rinder pest virus. Trop. Anim. Hlth. Prod., 17(2):75-81.
19. Smyth, J.A., M.A.Platten and J.B. Mcferran. 1988. A study of the pathogenesis of egg drop syndrome in laying hens .Avian pathol.,17 : 653-666.
20. Van ECK, J.H.H.,F.G. Davelear, T.A.M. Van den HePlesman, N. Van Kol, B.F.H.M.G. Kouwenhoven and F.H.M.G. 1976. Dropped egg production soft shelled and shell less eggs associated with appearance of precipitins to virus in flocks of laying fowl.Avian pathol., 5: 261-272.

برنامج تحصيني ضد مرض تدنى البيض في الدجاج باستخدام اللقاحات المحضرة محلياً

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معهد بحوث الامصال و اللقاحات بالعباسية- مركز البحوث الزراعية – وزارة الزراعة – الدقي – جيزة

تم خلال هذا العمل تطبيق عشرة برامج مختلفة للتحصين ضد ظاهرة تدنى البيض في الدجاج باستخدام ثلاثة صورللقاحات محضرة محلياً أشتملت على لقاحين نسيجيين (محضرين على أنسجة أجنة الدجاج) أحدهما حى مستضعف والآخر مثبط وثالث مثبط محضر فى أجنة البط حيث تم تحصين مجموعات من الكتاكيت عند عمر أربعة أسابيع وتسع عشر أسبوعا متضمنا ذلك التحصين بجرعة واحدة وبجرعة منشطة مع وجود ضوابط غير محصنة. وقد تم تقييم كفاءة البرامج في الدجاج المحصن بقياس المناعة الخلطيه بإجراء اختبارى المصل المتعادل ومنع التلازن الدموي وقياس متوسط إنتاج البيض ونوعيته حيث أظهرت النتائج أن أفضل البرامج هى أرقام ٤-٥-٦ التى يتم فيها تحصين الكتاكيت في العمر الصغير (٤ أسابيع) مع إعطاء جرعة منشطة قبل موسم وضع البيض باستخدام اللقاح المثبط والحي المستضعف ثم التحصين قبل موسم وضع البيض ويليها التحصين في السن الصغير فقط وتوصى الدراسة بضرورة تحصين الكتاكيت في السن الصغير وقبل موسم وضع البيض للحصول على أعلى نسبة لإنتاج البيض.