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Comparative immunological studies on some single and combined live attenuated vaccines in poultry

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In a trial for comparison between the efficiency of single fowl pox (FP) vaccination and the efficiency of each combined FP and Avian encephalomyelitis (AE) vaccination and simultaneous vaccination with FP and Reo and with FP and Chicken anemia virus (CAV) vaccines our conclusion was that there is no antagonistic reaction between FP virus strain and each AE, Reo and CA viruses strains. In addition, humoral immune response against AE virus strain in case of combined AE+FP vaccination is markedly potent than that in case of single AE vaccination, more over the value of average EID_{50} of AE virus strain in several batches of combined AE+FP vaccines is significantly higher at $P \geq 0.05$ than that in several batches of single AE vaccines. On the other hand, immune response against FP virus strain and Reo virus strain in case of simultaneous vaccination with FP and Reo vaccines is higher than that in case of single FP vaccination and single Reo vaccination. Consequently, it is advisable to use combined live attenuated AE+FP vaccine instead of vaccination with single FP and AE separately. Also, application of simultaneous vaccination with FP and Reo vaccines is advisable as it is proved to be more beneficial than vaccination with each vaccine separately specially in case of that FP vaccine of low potency.

Some viral poultry diseases cause very high rates of mortality or great decrease in production resulted in dramatic economic losses. However avian encephalomyelitis (AE) and fowl pox (FP) are among viral diseases that cause considerable economic losses to poultry due to the drop in egg production in laying hens and retarded growth in young chickens (Tripathy, 1989), Reovirus infection causes 100% morbidity (Frederick *et al.,* 1999). This disease causes economic losses as a result of crippling, viral arthritis, reduced marketability of the affected birds, diminished weight gain and poor food conversion (Dabson and Glisson, 1992). Also chicken anemia virus (CAV) infection causes immunosuppression thus it causes serious economic losses in commercial poultry production (Nova and Ragland, 2001) beside inadequate response to vaccination programs (Franz and Coral, 2003). Consequently vaccination against these viral diseases became necessary specially that using combined vaccines which are preferable as they have advantage of providing protection against more than one disease, reducing vaccination expense, saving time and labor costs besides reducing the stress reactions. Also, simultaneous vaccination help in improving the immune response against to vaccines which simultaneously applied especially if fowl pox vaccine included as it was known as an immunostimulant (Gergis *et al.,* 1994; Sherif *et al.,* 2002).

So, the objective of this study was to compare between the efficiency of single fowl pox vaccination and the efficiency of each combined AE+FP vaccination; simultaneous vaccination with FP and Reo vaccine and simultaneous vaccination with FP and CAV vaccine, in addition to determine the possibility of interference or antagonistic reactions between the two viruses antigens in combined and simultaneous vaccination through the following: (1) Estimation of the egg infective dose fifty (EID_{50}) for AE and FP virus strain in several batches of single AE and FP vaccines in comparison with that in several batches of combined AE+FP vaccines using SPF embryonated chicken eggs (ECE), (2) Investigation of immune response against FP virus in different vaccinated chickens groups by detecting the percentage of chickens showing FP post vaccination lesions (takes) and protection percentage in each group post challenge with virulent FP virus, (3) Evaluation of chicken humeral immune response to AE, Reo and CA viruses in chickens vaccinated groups by application of commercial ELISA kit for

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detection of antibodies against AE, Reo and CAV.

Materials and methods

SPF embryonated chicken eggs (ECE). 1560, 12 days old SPF ECE were used for titration of fowl pox vaccines and 2550, 6 days old SPF ECE were used for titration of AE vaccines. These eggs were supplied by Koum Osheim SPF Farm, Fayoum Governorate, Egypt.

Chickens. Two hundreds and ten (210) SPF chickens of 6 weeks old(sutable age for FP" AE "Reo and CAV vaccination according to the manufacturer instruction) were obtained from SPF Farm, Koum Osheim, Fayoum governorate, Egypt and reared under hygienic measures in isolated cages.

Vaccines. These vaccines include 5 commercial imported ready prepared vaccines.

Live attenuated strain of fowl pox virus was in two different vaccines.

FP vaccine of EID_{50}/dose > $10^{4.2}$ (Intervet Co.).

FP vaccine of its EID_{50}/dose was $10^{2.8}$ (Intervet Co.).

Live attenuated strain of AE virus of EID_{50}/dose $10^{3.0}$ (IZO Co.).

Live attenuated Reovirus of $TCID_{50}$/dose $10^{4.0}$ (Intervet Co.).

Live attenuated CAV of $TCID_{50}$/dose $10^{3.6}$ (Intervet Co.).

Combined bivalent live attenuated AE and FP vaccine of EID_{50}/dose $10^{4.2}$ for FP virus and EID_{50}/dose $10^{3.7}$ for AE virus (Intervet Co.).

Virulent strain. Egyptian virulent FP virus was used as challenge virus of a titre $10^{6.0}$ EID_{50}/ml and used in a dose of $10^{3.0}$ EID_{50}/bird. It was isolated and identified by (Saban, 1954).

Experimental Design. Chickens were divided into eight groups as follow:

Group (1). Consisted of 45 chickens divided into two subgroups:

Subgroup (1A). Containing 15 chickens were vaccinated with single FP vaccine of EID50/dose $>10^{4.2}$ via the wing web route in the right wing.

Subgroup (1B). Consisted of 30 chickens were vaccinated with single FP vaccine of EID50 /dose$10^{2.8}$ through the same route in subgroup 1A.

Group (2). Containing 15 chickens were vaccinated with live attenuated AE vaccine through drinking water.

Group (3). Containing 30 chickens were vaccinated with combined bivalent attenuated FP and AE vaccine via the wing web in right wing.

Group (4). Consisted of 15 chickens were vaccinated with live attenuated Reovirus vaccine in a dose of 0.2 ml/bird through the subcutenious (s/c) rout

Group (5). Thirty chickens were vaccinated simultaneously with live attenuated Reo vaccine(0.2ml/bird s/c)with FP vaccine(of $10^{2.8}$ EID_{50}/dose) via wing web route in right wing.

Group (6). Fifteen chickens were vaccinated with live attenuated CAV vaccine using 0.5ml/bird inoculated intramuscularly (IM).

Group (7). Thirty chickens were vaccinated simultaneously with CAV vaccine(using 0.5mi/bird inoculated IM) withFP vaccine (of $>10^{2.8}$ EID_{50}/dose) through wing web in the right wing.

Group (8). Thirty chickens were kept unvaccinated in separate cages as negative control birds.

The vaccines were administered as recommended by manufacturer instruction.

Samples. Ten random blood samples were collected from each chicken group weekly allover the experimental period (8-10 weeks). The obtained serum samples were tested for evaluation of the humoral immune response against AE virus in groups 2 and 3, Reovirus in groups 4 and 5 and CAV in groups 6 and 7 Using ELISA.

Enzyme linked immunosorbent assay (ELISA). ELISA kits for AE (catalog No. CK 123), for Reovirus (catalog No. CK 110) and for CAV (catalog No. 126) were supplied by Biocheck Co., Holland.

ELISA for detection of antibodies against AE, Reo and CAV was carried out according to (Sharen and Tanock, 1988), (Giambrone *et al.,* 1991), and (Myrna *et al.,* 2003) respectively

Determination of percentage of chickens showing takes. It was carried out by examination of the site of FP vaccination (right wing web) in each group including groups of chickens vaccinated with FP, combined FP+AE (groups 1 and 3 respectively) and groups simultaneously vaccinated with FP+Reo and FP+CAV (groups 5 and 7, respectively) (According to Code of Federal Regulations, 2006).

Challenge test. Three weeks post vaccination, ten chickens from each vaccinated groups 1, 3, 5 and 7 and control group 8 were challenged with standard challenge dose of virulent fowl pox virus containing $10^{3.0}$ EID_{50}/bird through wing web in the left wing, then the challenged birds were checked for takes at 10th and 14th day post challenge.

Titration of single and combined bivalent vaccines of FP and AE virus. Titration of AE vaccines (single AE and combined AE+FP) was done according to Code of Federal Regulations (2006) in 6 day old SPF ECE through the intra-yolk route. At the third day from the beginning of hatching, hatched chicks in each dilution were examined for any symptoms related to AE virus and the EID_{50} was calculated according to Reed and Muench (1938). Titration of FP vaccines (single FP and combined FP+AE) was carried out in five 12 days old SPF ECE on chorioallantoic membrane (CAM). CAMs of eggs were examined at the 7th day post inoculation for presence of pock lesions in each dilution. EID_{50} was calculated according to (Reed and Muench,1938).

Results and Discussion

Table (1) showed that the average $\log_{10}$ of EID_{50} of AE virus strain in fifteen batches of combined AE and FP vaccines was 3.92 which is significantly higher at $P \geq 0.05$ than that in twenty two batches of single AE vaccines (3.29). In addition, AE ELISA antibody geometric mean titre (GMT) in combined AE and FP vaccinated chickens (group 3) was markedly higher than the corresponding GMTs in the single AE vaccinated (group 2) through the ten week post vaccination (WPV) (Table 2 and Fig. 1).

It is clear that the humoral immune response against AE virus strain and the EID_{50} of AE virus strain in case of combined AE and FP vaccination is higher than that in case of single AE vaccination. This finding could be attributed to the immune stimulant effect of FP virus is in agreement with that obtained by Gergis *et al.,* (1994) and Sherif *et al.,* (2002).

On the other hand, percentage of chickens showing FP vaccination lesions in combined AE and FP vaccinated chickens (group 3) was 91.6% which was nearly equal or slightly lower than that in single FP vaccinated group (1A) (100%) as shown in Table (5).

These findings comes parallel to those in table (6) which showed that the protection percentage at 2nd week post challenge with virulent FP virus at the 3rd WPV in combined AE+FP vaccinated group (3) was slightly lower (90%) than that in single FP vaccinated group (1A) (100%). In addition, table (1) explains that the average EID_{50} of FP strain in 27 batches of single FP vaccines and in fifteen batches of combined AE+FP vaccines is nearly equal as they are 3.81 and 3.84, respectively. This previous findings indicates that the protection percent and percent of chicken showing FP vaccination lesion and EID_{50} of FP strain in case of combined AE+FP vaccination is nearly equal to that in case of single FP vaccination.

In conclusion, there is no antagonistic reaction between the two antigens AE and FP when combined as live vaccine. Moreover, FP acts as immunostimulant to AE virus. This conclusion encouraging application of combined vaccination in the field as combined vaccines has many advantages than single one (Abdel Wanis *et al.,* 1999; Afaf *et al.,* 1999 and Sherif *et al.,* 2002).

In simultaneous vaccination, experiment at first we used FP vaccine with $>10^{4.2}$ EID_{50}/dose for vaccination of 3 groups of chickens (single FP vaccinated group, FP and Reo vaccinated group, FP and CAV vaccinated group). The percentage of chickens showing takes and the protection percentage against FP challenge virus were the same in the above 3 groups (100%). So, we repeated this experiment using another FP vaccine with $10^{2.8}$ EID_{50}/dose which gave 70% protection in FP vaccinated chickens to enable us to differentiate between the above three groups. Table (5) illustrated that the The percentage of chickens showing FP vaccination lesions is 100% in simultaneously vaccinated group (5) with FP of $10^{2.8}$ EID_{50}/dose and Reo. This percentage is higher than that in single FP vaccinated subgroup (1B) (it is 75%). This result is in agreement with that in table (6) which illustrated that protection percentage at 2nd week post challenge with virulent FP virus at 3rd WPV in the FP and Reo simultaneously vaccinated group (5) is 100% higher than that in the single FP vaccinated subgroup (1B) (70%).

We repeat this challenge experiment twice and the same result was obtained on the other hand, table (3) showed that Reo ELISA antibody GMTs in single Reo vaccinated chickens (group 4) are slightly lower than that in simultaneous vaccinated chickens with FP and Reo vaccines (group 5) through the nine WPV. This titre in the two groups was protective (Thayer *et al.,* 1986). The explanation of these findings showing that there is synergism between Reo and FP virus strains if used together in simultaneous vaccination. As the humoral immune response against Reovirus strain, percentage of chickens showing FP vaccination lesions and protection percentage against FP challenge are higher in FP and Reo simultaneously vaccinated group than that in single FP and single Reo vaccinated group. In addition, there is no antagonistic

Table (1): Titres of AE and FP viruses (Log_{10} EID_{50}) in several batches of single and combined AE and FP vaccines.

Vaccine type	Virus Titre			
	Single FP	Single AE	Combined AE+FP	
			FP	AE
Average Log_{10} EID_{50}/dose	3.81	3.29 + 0.37	3.84	3.92+0.274
No. of vaccine batches	27	22	15	

FP Fowl Pox.
AE Avian Encephalomyelitis.
EID_{50} Egg Infective Dose fifty.
SPF Specific Pathogen Free.

Table (2): AE ELISA antibody GMTs in chickens vaccinated with single AE vaccine and combined AE and FP vaccines.

Weeks post vaccination	ELISA antibody titre GMT in chicken group		
	Group (2)	Group (3)	Group (8)
1	211	290	201
2	241	343	355
3	310	679	311
4	625	920	320
5	807	949	331
6	1100	1200	357
7	1276	1317	318
8	1121	1617	320
9	1120	2025	325
10	930	2250	326

Group (2): Chickens vaccinated with single AE vaccine.
Group (3): Chickens vaccinated with combined AE and FP vaccine.
Group (8): Control unvaccinated chickens.
GMT: Geometric mean titres.
Log_{10} titre = 1.1 (Log_{10} SP) + 3.361.
Titre = Anti-Log 10^x.
Titre of positive serum sample was $\geq$ 1071.
Pre-vaccination AE ELISA antibody GMT = 106.

reaction between FP and Reo when inoculated simultaneously in chickens as Reo vaccine not interferes with many types of avian vaccines (Edison and Kleven, 1983, Giambrone and Hathcoock, 1991). Moreover, Reo vaccination improves the immune response against FP vaccination. This result encourages the application of simultaneous vaccination with Reo and FP vaccines specially in case of FP vaccines of low potency.

Table (4) explained that CAV ELISA antibody GMTs in simultaneously vaccinated chickens with FP and CAV vaccines (group 7) are slightly higher than the corresponding GMTs in single CAV vaccinated group (6) through the nines WPV. All titres in these groups were protective (Malo and Weingartan, 1995). On the other hand, percentage of chickens showing FP vaccination lesions in FP and CAV simultaneously vaccinated group (7) is 83.3 % which is slightly higher than that in single low titre FP vaccinated group (1b) (75%) (Table 5). This result is parallel to that in table (6) which showed that protection % against FP challenge in simultaneously vaccinated group (7) with FP and CAV is 80% slightly higher than that in single FP vaccinated group (6) (it is 70%).

It is concluded that there is no antagonistic reaction between FP and CAV virus strain if used in vaccination simultaneously. Moreover, FP stimulated slightly humoral immune response against CAV vaccine. On the other hand, live attenuated CAV vaccine is safe if inoculated simultaneously with FP vaccine and not cause immunosuppression (Hanan *et al.,* 2008). Also, CAV vaccine not reverse to its virulence as mentioned by Todd *et al.,* (1995, 1998).so, it could be concluded that FP virus strain not has antagonistic reaction with AE, Reo or CA virus strains. Moreover, FP virus stimulates the

immune response against AE, Reo and CAV virus strains.

Table (3): Reo ELISA antibody GMTs in chickens vaccinated with Reo vaccine and simultaneously with FP and Reo vaccines.

Weeks post vaccination	ELISA antibody GMT of chicken group		
	Group (4)	Group (5)	Group (8)
2	4000	3390	464
3	5040	5108	ND
4	7800	7890	786
5	7144	7472	ND
8	5774	7380	642
9	6154	8371	ND

Group (4): Chickens vaccinated with single Reo vaccine.
Group (5): Chickens simultaneously vaccinated with Reo and FP vaccines.
Group (8): Control unvaccinated chickens.
GMT: Geometric mean titres.
Log_{10} titre = 1.1 (Log_{10} SP) + 3.9.
Titre = Anti-Log 10^{x}.
Titre of positive serum sample was $\geq$ 1352.
Pre-vaccination Reo ELISA antibody GMT = 1120.

Table (4): CAV ELISA antibody GMTs in chickens vaccinated with single CAV and simultaneously with CAV and FP vaccines.

Weeks post vaccination	ELISA antibody GMT of chicken group		
	Group (6)	Group (7)	Group (8)
2	2012	3672	186
4	3099	3947	193
6	4120	4327	ND
7	4662	4709	347
8	4781	4959	ND
9	7050	7523	366

Group (6): Chickens vaccinated with single CAV vaccine.
Group (7): Chickens simultaneously vaccinated with CAV and FP vaccines.
Group (8): Control unvaccinated chickens.
GMT: Geometric mean titres.
Log_{10} titre = 1.10 (Log_{10} SP) + 3.361.
Titre = Anti-Log 10^{x}.
Titre of positive serum sample was $\geq$ 724.

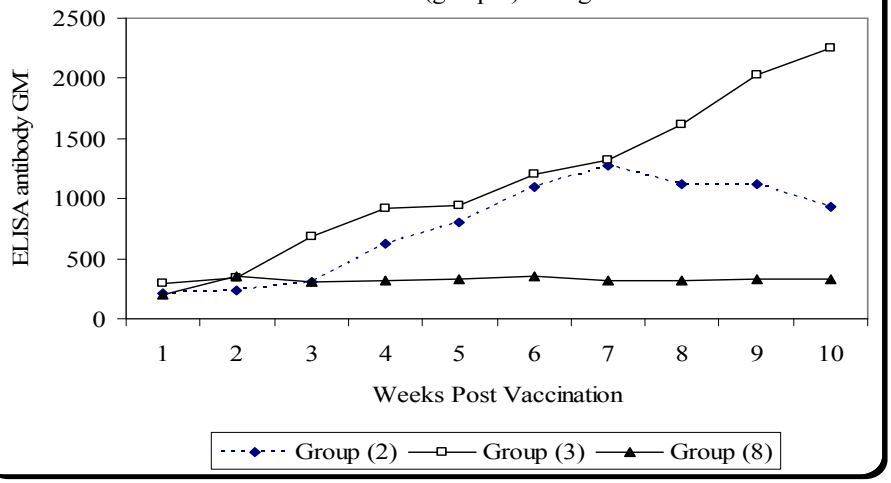
Table (5): Percentage of chickens showing FP vaccination lesion (takes) in single FP vaccinated sub group (1A and 1B), Reo and FP simultaneously vaccinated group, CAV and FP simultaneously vaccinated group and in combined AE and FP vaccinated group.

Chicken groups		Types of vaccines/method of vaccination	No. of +ve chickens/Total No.	% of chickens showing takes at 10^{th} DPV
1	**1A**	Single high titre FP	12/12	100 %
	1B	Single low titre FP	9/12	75 %
5		Simultaneously vaccinated with Reo and FP	12/12	100 %
7		Simultaneously vaccinated with CAV and FP	10/12	83.3 %
3		Combined AE and FP	11/12	91.6 %

Subgroup (1A): Chickens vaccinated with single FP vaccine with EID_{50}/dose = > $10^{4.2}$ (high titre FP vaccine).
Subgroup (1B): Chickens vaccinated with single live attenuated FP vaccine with EID_{50}/dose = $10^{2.8}$ (low titre FP vaccine).
Group (5): Chickens simultaneously vaccinated with live attenuated Reo vaccine and FP vaccine which used in subgroup 1B.
Group (7): Chickens simultaneously vaccinated with live attenuated CAV vaccine and FP vaccine which used in subgroup 1B.
Group (3): Chickens vaccinated with combined bivalent live attenuated AE+FP vaccine.

* +ve chickens: Chickens showing FP vaccination lesions (takes) at site of vaccination (right wing).

Fig. (1): AE ELISA antibody GMTs in chickens vaccinated with single AE vaccine (group 2) and in chickens vaccinated with combined AE and FP vaccine (group 3) through the tens WPV



Reo ELISA antibody GMTs in chickens vaccinated with Reo vaccine (Group 4) and simultaneously with FP and Reo vaccine (Group 5) through the nines WPV

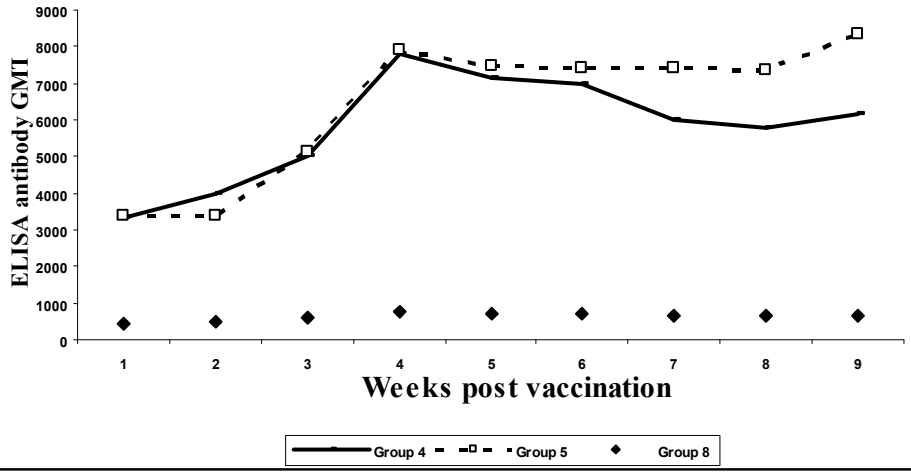


Fig. (3): CAV ELISA antibody GMTs in chickens vaccinated with single CAV vaccine (group 6) and in chickens simultaneously vaccinated with CAV and FP vaccines (group 7) through the nines WPV

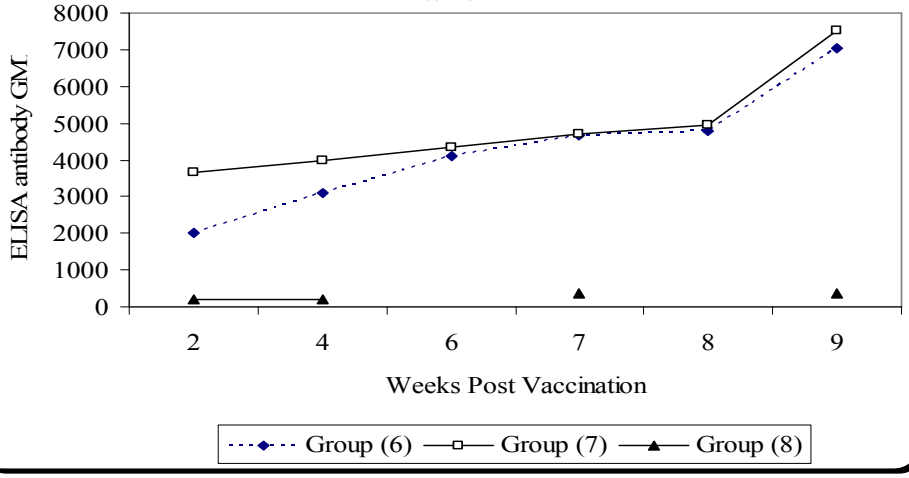


Table (6): Protection percentage at 2nd week post challenge with virulent FP virus at 3rd WPV in single FP vaccinated subgroup 1Aand 1B, Reo and FP simultaneously vaccinated group , CAV and FP simultaneously vaccinated group and combined AE and FP vaccinated group

Chicken groups	Types of vaccines/method of vaccination	No. of +ve chickens/Total No.	% of chickens showing takes at 10th DPV
1 **1A**	Single high titre FP	10/10	100 %
1B	Single low titre FP	7/10	70 %
5	Simultaneously vaccinated with Reo and FP	10/10	100 %
7	Simultaneously vaccinated with CAV and FP	8/10	80 %
3	Combined AE and FP	9/10	90 %
8	Control unvaccinated	0/10	0 %

Subgroup (1A): Chickens vaccinated with single FP vaccine its EID_{50}/dose = > $10^{4.2}$ (high titre FP vaccine).
Subgroup (1B): Chickens vaccinated with single live attenuated FP vaccine its EID_{50}/dose = $10^{2.8}$ (low titre FP vaccine).
Group (5): Chickens simultaneously vaccinated with live attenuated Reo vaccine and FP vaccine which used in subgroup 1B.
Group (7): Chickens simultaneously vaccinated with live attenuated CAV vaccine and FP vaccine which used in subgroup 1B.
Group (3): Chickens vaccinated with combined bivalent live attenuated AE+FP vaccine.
Group (8): Control unvaccinated chickens.
* +ve chickens: Chickens showing symptoms related to FP infection or challenge lesions (takes) at site of challenge (left wing).

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دراسة مناعية مقارنة على بعض اللقاحات الحية المستضعفة الأحادية والمركبة فى الدجاج

فى محاوله للمقارنه بين كفاءه التحصين باللقاح الاحادى لجدرى الطيور و كفاءه كلا من التحصين باللقاح المركب لجدرى الطيور و الارتعاش الوبائى والتحصين المتزامن بلقاح الجدرى مع الريو و لقاح الجدرى مع أنيميا الدجاج نخلص من هذه الدراسه الى عدم وجود أى تفاعلات متداخلة أوتعارض بين العترة الفيروسية لجدرى الطيور وكلاً من العترة الفيروسية للارتعاش الوبائى والريو وانيميا الدجاج ، كما وجد أن رد الفعل المناعى ضد عترة فيروس الارتعاش الوبائى فى حالة التحصين بلقاح جدرى الطيور والارتعاش الوبائى المركب اقوى بصورة واضحة من ذلك الموجود فى حالة التحصين بلقاح الارتعاش الوبائى الأحادى و زياده على ذلك قيمة متوسط نصف الجرعة المعدية للبيض الخاص بعترة فيروس الارتعاش الوبائى للعديد من دفعات اللقاحات المركبه لجدرى الطيور والارتعاش الوبائى تزيد زيادة معنوية عند تلك القيمة للعديد من دفعات اللقاحات الاحاديه للارتعاش الوبائى . من ناحية أخرى وجد ان الأستجابه المناعيه ضد فيروس جدرى الطيور و فيروس الريو فى حالة التحصين المتزامن للقاح جدرى الطيور مع الريو اعلى من تلك اللأستجابه المناعيه فى حالة التحصين بلقاح جدرى الطيور الأحادى و التحصين بلقاح الريو الأحادى ، لذا يوصى باستخدام اللقاح المركب لفيروس جدرى الطيور والارتعاش الوبائى بدلاً من التحصين بكل لقاح على حدة. كما يوصى بتطبيق التحصين المتزامن للقاح جدرى الطيور مع الريو حيث أنه أثبت تفوقه على التحصين بكل لقاح على حدة وبالأخص فى حالة لقاحات جدرى الطيور ضعيفة القوة العيارية.