



COMPARATIVE STUDY ON LIVE ATTENUATED AND INACTIVATED CHICKEN ANEMIA VIRUS VACCINES

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ABSTRACT

The aim of this research was to compare and evaluate the potency and safety of live and inactivated vaccines. This has been investigated by using 180 specific pathogen-free (SPF) chickens. 100 chickens were given the live vaccine, 50 were given the inactivated vaccine and 30 were used as non-vaccinated control. The antibody titer was measured periodically until 40 weeks post vaccination using ELISA and SNT. The results showed that the live CAV vaccinated birds exhibited detectable levels of specific CAV antibodies by the 1st week recording peak titers by the 8th week post vaccination. In contact non-vaccinated birds attracted the excreted virus from vaccinated chickens and exhibited lower antibody titers. Chickens vaccinated with inactivated CAV vaccine showed detectable specific CAV antibodies by 1st week post vaccination recorded that the peak titer for both groups by the 8th week post vaccination. These titers began to decline by the 32nd week post vaccination. Away control non-vaccinated chickens remain sero-negative in both groups. Evaluation of the hematocrit values in chickens vaccinated with the live CAV vaccine showed decreased levels by the 2nd week post vaccination then began to return to safe levels started by the 3rd week. On the other side all chicken groups vaccinated with the inactivated CAV vaccine did not show decline in their hematocrit values as well as in contact and away control non-vaccinated chickens confirmed the complete safety of such vaccine. We concluded from this study that the inactivated CAV vaccine was highly immunogenic and safer than the live vaccine.

Keywords: Chicken anemia vaccine, ELISA, SNT

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1. INTRODUCTION

CAV is a ubiquitous and highly resistant virus of chickens that causes anemia and death in chicks less than 3 weeks of age and immunosuppressant in chickens older than 3 weeks of age [1]. It was recorded in different countries all over the world [2]. In Egypt, CAV has been suspected since long time based on clinical symptoms and post mortem lesions in the major poultry raising states of the country [3] and [4]. CAV infection is characterized by clinical and subclinical infection. The disease is wide spread in breeder and commercial chicken

flocks [1]. The present study aims to prepare and evaluate in comparison live and inactivated CAV vaccines.

2. MATERIALS AND METHODS

2.1. Virus strain:

Commercial chicken anemia virus (CAV) vaccine adapted and propagated on MDCC cell line was kindly supplied by Inter Vet Company. CAV-VAC is live virus vaccine prepared from a modified US field isolate of chicken anemia virus (CAV).

2.2. Virus titration.

Titration for the propagated CAV in VERO cell culture was carried out using the micro-titer technique [5] and the virus titer was calculated as log₁₀ TCID₅₀/ml [6].

2.3. African green monkey kidney cell line (VERO).

It was kindly supplied by the Department of Pet Animal Vaccine Research; Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

2.4. Montanoid oil ISA-70 VG.

It was obtained from SEPPIC, Cosmetics, pharmacy Division, Paris, France. It was used as an adjuvant for the inactivated CAV vaccine.

2.5. Preparation of live attenuated CAV vaccine:

The CAV pool obtained from Vero cell culture was freeze-thawed three times, and centrifuged at 15000 rpm, for 20 min, to eliminate the cell debris. 20% skimmed milk was added to the virus suspension with thoroughly mixing then dispensed in neutral glass vials and subjected to freeze drying lyophilization [7].

2.6. Preparation of inactivated CAV vaccine.

Inactivation of the propagated virus suspension with 0.2% formalin and incubation at 37°C for 72 h. The complete inactivation of the CAV virus was checked by making 3 serial passages in cell culture, and checking the absence of virus by immune fluorescence [8]. The resulting inactivated virus was adjuvant with Montanide ISA-70 oil adjuvant added 3:7 to the inactivated CAV suspensions according to the protocol of SEPPIC Pharmacy Division, France. (30 gm aqueous antigenic media & 70 gm montanoid TM ISA 70 VG).

2.7. Experimental Chicks.

180 SPF 3months age hens were purchased from El-Fayoum from SPF farm. These

chicks were housed under strict hygienic measures and used for evaluation of the prepared vaccine. These chickens were grouped as demonstrated in table (1).

Table 1. Schedule of chicken vaccination

Chicken groups	Used vaccine	Number of chicken	Dose
1	Live	25	0.5ml (4log ₁₀ TCID ₅₀)
2	Live	25	0.5ml (3log ₁₀ TCID ₅₀)
3	Live	25	0.5ml (2log ₁₀ TCID ₅₀)
4	Live	25	0.5ml (log ₁₀ TCID ₅₀)
5	Inactivated	25	0.5ml
6	Inactivated	25	1.0ml
7.1	contact with live	10	-
7.2	contact with inactivated	10	-
7.3	Non-contact	10	-

2.8. Serum samples.

Serum samples were collected from all chicks (vaccinated and non- vaccinated) weekly till 40weeks post vaccination for monitoring of CAV antibody titer using ELISA and SNT.

2.9. Blood samples.

Blood samples were collected from all chicks (vaccinated and non- vaccinated) weekly till 4th week post vaccination to evaluate the hematocrit values.

2.10. Quality control tests of the prepared CAV vaccines.

Safety and sterility tests were carried out on experimental samples of the prepared vaccines [9].

2.11. Serum neutralization test (SNT).

SNT was carried out using the micro titer technique [10] for evaluation of humeral immune response for prepared vaccine . The antibody titer was expressed as the reciprocal of the final serum dilution which neutralized and inhibited completely the CPE of 100 TCID₅₀ of the used virus [11].

2.12. ELISA Technique.

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The technique was performed using ELISA test kit for detection of CAV antibody was supplied by Synbiotics, USA, Serial No.2UCCAV 45001 according to the manufacturer instruction. A CAV positive control serum has been provided with kit. The average normal control absorbance was subtracted from the average positive absorbance. The difference is the corrected positive control. A sample to positive (SP) ratio was calculated by subtracting the average normal control serum absorbance from each sample absorbance. The difference was divided by the corrected positive control. The following equation format was used:

$$SP = \frac{\text{Sample absorbance} - \text{Average normal control absorbance}}{\text{Corrected positive control absorbance}}$$

2.13. Evaluation of hematocrit value.

Blood samples were collected from jugular vein of chickens on transferring tubes containing 6% EDTA solution. Blood was then transferred to microhematocrit capillary tubes (Scientific products, McGraw Park, III). PCVs were determined by centrifugation of the Microhematocrit capillary tube, measuring the PCV, and recording PCV values [12].

3. RESULTS

3.1. Sterility and Safety of the prepared vaccines.

The prepared cell culture live and inactivated CAV vaccines were found to be free from bacterial (aerobic and anaerobic); fungal and mycoplasma contaminations. Regarding the safety of live CAV vaccine; it was found that 15 out of 25 (60%) and 10 out of 25 (40%) chickens which was vaccinated using doses of 4 and 3 log₁₀TCID₅₀/bird, respectively, showed depletion, off food and pale mucous membranes with decreased body weight. Birds received live CAV vaccine in doses (2- and 1 log₁₀TCID₅₀/bird) did not show any abnormalities. Eight in contact chickens

showed low titer of CAV antibodies as demonstrated by SNT and ELISA while 2 birds showed sever clinical signs. On the other side using double doses of the inactivated vaccine showed no post vaccinal reactions among all vaccinated chickens and in contact controls where all of them remained healthy all over the experimental period and no virus recovery was recorded. In contact chickens did not exhibited any detectable CAV antibodies revealing the safety of the inactivated vaccine. These results were shown in table (2).

Table 2. Safety of live attenuated CAV vaccine.

Chicken groups	Number of chickens	Number of chickens showing symptoms	Percentage Of Safety (%)
1	25	15	40
2	25	10	60
3	25	0	100
4	25	0	100
7.1	10	2	80
7.3	10	0	100

Group-1: Vaccinated with 4log₁₀TCID₅₀/bird,
 Group-2: Vaccinated with 3log₁₀TCID₅₀/bird.
 Group-3: Vaccinated with 2log₁₀TCID₅₀/bird,
 Group-4: Vaccinated with 1log₁₀TCID₅₀/bird.
 Group-7.1: Non-vaccinated in contact.
 Group-7.3: Non-vaccinated away chickens.

3.2. Evaluation of humoral immune response for prepared vaccine.

Potency of the prepared live CAV vaccine was evaluated through monitoring of induced antibodies in the sera of vaccinated chickens. It was noticed that vaccinated birds with high doses 3 and 4 log₁₀TCID₅₀ exhibited detectable levels of specific CAV antibodies by the 1st week (8 by SNT, and 2798 and 2338 by ELISA) recording peak titers (128 by SNT, and 12410 and 11720 by ELISA) by the 8th week post vaccination. Chickens receiving lower doses 2 and 1 log₁₀TCID₅₀ showed lower antibody titers (4 and 2 by SNT, and 2338 and 1774 by ELISA) on the 1st week with peak titers on the 12th week post

vaccination (32 and 16 by SNT, and 9527 and 8992 by ELISA).

In contact non-vaccinated birds attracted the excreted virus from vaccinated chickens and exhibited lower antibody titers (2 by SNT and 2238 by ELISA) on the 2nd week recording peak titer (8 by SNT and 3639 by ELISA) by the 12th week.

Away control non-vaccinated chickens remain sero-negative all over 40 weeks. However, the recorded levels of antibodies began to decrease by the 36th week post vaccination although they remain high within group 1 and 2. These results are showed in tables (3 and 4) and graphs (1 and 2).

Potency of the prepared inactivated CAV vaccine showed that both of group-5 and group-6 vaccinated either with 0.5ml or 1.0ml of the inactivated CAV vaccine showed detectable specific CAV antibodies by 1st week post vaccination (4 and 8 by SNT, and 2885 and 3491 by ELISA, respectively) by the 1st week recorded the peak titer for both groups (128 by SNT, and

12697 and 11311 by ELISA, respectively) by the 8th week post vaccination. These titers began to decline by the 32nd week post vaccination. In contact (group-7.2) and away control birds remain sero-negative all over the experimental period as demonstrated in tables (6 and 7) and graphs (3 and 4).

3.3. Evaluation of the hematocrit values in vaccinated chickens.

Evaluation of the hematocrit values in chickens vaccinated with the live CAV vaccine showed decreased levels in group 1, 2 and 3 to reach anemic levels by the 2nd week post vaccination then began to return to safe levels started by the 3rd week. Group 4 and 7.1 did not show significant decline in such values while group 7.3 remain within the normal level as shown in table (5). On the other side all chicken groups vaccinated with the inactivated CAV vaccine did not show decline in their hematocrit values as well as in contact and away control non-vaccinated chickens as tabulated in table (8).

Table 3. Mean neutralizing antibody titers in chickens vaccinated with the live CAV vaccine

Weeks post vaccination	Mean CAV neutralizing antibody titer*					
	Group-1	Group-2	Group-3	Group-4	Group-7.1	Group-7.3
0	0	0	0	0	0	0
1	8	8	4	2	0	0
2	16	16	8	2	> 2	0
3	32	16	8	4	2	0
4	64	32	16	8	4	0
8	128	128	16	8	4	0
12	128	128	32	16	8	0
16	128	128	32	16	8	0
20	128	128	32	16	8	0
24	128	128	32	16	4	0
28	128	128	32	16	8	0
32	128	128	32	8	8	0
36	128	128	16	8	2	0
40	64	64	16	8	2	0

*Antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 TCID₅₀ of CAV. Group-1: Vaccinated with 4log₁₀TCID₅₀/bird, Group-2: Vaccinated with log₁₀TCID₅₀/bird. Group-3: Vaccinated with 2log₁₀TCID₅₀/bird, Group-4: Vaccinated with 1log₁₀TCID₅₀/bird. Group-7.1: Non-vaccinated in contact. Group-7.3: Non-vaccinated away chickens

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Table 4. Mean ELISA antibody titer of CAV in chickens vaccinated with the live CAV vaccine.

Weeks post vaccination	Mean ELISA titer of CAV antibodies					
	Group-1	Group-2	Group-3	Group-4	Group-7.1	Group-7.3
0	1854	1849	1839	1860	1819	1475
1	2798	2338	2338	1774	1902	1510
2	3060	3798	2727	2238	2238	1514
3	5436	4099	3311	2335	2853	1512
4	5958	6849	4533	4928	2725	1510
8	12410	11720	5696	5969	2951	1511
12	11749	11491	9527	8992	3639	1512
16	11930	11087	9226	6958	3454	1513
20	11839	11600	9270	6884	3396	1515
24	11975	11719	9048	6811	3426	1495
28	11952	11620	8509	6567	3546	1504
32	11895	11660	8557	6762	3442	1490
36	10387	10420	6413	5664	3338	1510
40	6653	6925	10425	7420	3144	1500

Group-1: Vaccinated with $4\log_{10}\text{TCID}_{50}/\text{bird}$, Group-2: Vaccinated with $3\log_{10}\text{TCID}_{50}/\text{bird}$. Group-3: Vaccinated with $2\log_{10}\text{TCID}_{50}/\text{bird}$, Group-4: Vaccinated with $1\log_{10}\text{TCID}_{50}/\text{bird}$. Group-7.1: Non-vaccinated in contact. Group-7.3: Non-vaccinated away chickens

Table 5. Hematocrit values in chickens vaccinated with live CAV vaccine.

Week post vaccination	Hematocrit Values are mean of five chicks per group					
	Group-1	Group-2	Group-3	Group-4	Group-7.1	Group-7.3
0	32.6	28.6	28.2	32.0	31.1	32.5
1	29.6	26.2	27.7	30.0	30.0	32.6
2	20.8	19.6	20.8	29.6	25.2	32.6
3	27.7	26.4	28.2	31.0	26.2	32.5
4	30.2	31.0	32.4	32.0	28.4	32.6

Hematocrit value < 26 is considered to be anemic. Group-1: Vaccinated with $4\log_{10}\text{TCID}_{50}/\text{bird}$, Group-2: Vaccinated with $3\log_{10}\text{TCID}_{50}/\text{bird}$. Group-3: Vaccinated with $2\log_{10}\text{TCID}_{50}/\text{bird}$, Group-4: Vaccinated with $1\log_{10}\text{TCID}_{50}/\text{bird}$. Group-7.1: Non-vaccinated in contact. Group-7.3: Non-vaccinated away chickens

Table 6. Mean neutralizing antibody titers in chickens vaccinated with inactivated CAV vaccine.

Weeks post vaccination	Mean CAV neutralizing antibody titer*			
	Group-5	Group-6	Group-7.2	Group-7.3
0	0	0	0	0
1	4	8	0	0
2	16	16	0	0
3	32	32	0	0
4	64	64	0	0
8	128	128	0	0
12	128	128	0	0
16	128	128	0	0
20	128	128	0	0
24	128	128	0	0
28	128	128	0	0
32	128	128	0	0
36	32	32	0	0
40	16	16	0	0

*Antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 TCID_{50} of CAV. Group-5: Vaccinated with $0.5\text{ml}/\text{bird}$ of inactivated CAV vaccine, S/C. Group-6: Vaccinated with $0.1\text{ml}/\text{bird}$ of inactivated CAV vaccine, S/C. Group-7.2: Non-vaccinated in contact. Group-7.3: Non-vaccinated away chickens

Table 7. Mean ELISA titer of CAV antibodies in chickens vaccinated with the cell culture inactivated CAV vaccine.

Weeks post vaccination	Mean CAV antibody titer*			
	Group-5	Group-6	Group-7.2	Group-7.3
0	1819	1080	1487	1475
1	2885	3491	1498	1510
2	3491	4253	1468	1514
3	4350	6892	1500	1512
4	6892	7134	1437	1510
8	12697	11376	1473	1511
12	10715	11311	1475	1512
16	10248	11297	1492	1513
20	10232	10665	1490	1515
24	10333	10601	1402	1495
28	10434	10556	1452	1504
32	9340	10120	1463	1490
36	8789	9932	1481	1510
40	8542	8925	1478	1500

Group-5: Vaccinated with 0.5ml/bird of inactivated CAV vaccine, S/C. Group-6: Vaccinated with 0.1ml/bird of inactivated CAV vaccine, S/C. Group-7.2: Non-vaccinated in contact. Group-7.3: Non-vaccinated away chickens

Table 8. Haematocrite values in chickens vaccinated with inactivated CAV vaccine

Week post vaccination	Hematocrit Values are mean of five chicks per group			
	Group-5	Group-6	Group-7.2	Group-7.3
0	32.5	32.1	31.8	32.5
1	31.9	31.8	32.1	32.6
2	32.4	32.2	31.9	32.6
3	32.5	32.1	31.8	32.5
4	32.4	32.0	32.0	32.6

Hematocrit value < 26 is considered anemic. Group-5: Vaccinated with 0.5ml/bird of inactivated CAV vaccine, S/C. Group-6: Vaccinated with 0.1ml/bird of inactivated CAV vaccine, S/C. Group-7.2: Non-vaccinated in contact. Group-7.3: Non-vaccinated away chickens

4. DISCUSSION

Safety of CAV vaccines showed that live CAV vaccine is of low safety while inactivated CAV vaccine revealed high safety, with no post vaccinal reactions among all vaccinated chickens and in contact

controls that were remained healthy all over the experimental period and no virus recovery was recorded. These results come in agreement with the study, which demonstrated that the use of an inactivated vaccine for CAV has obvious advantages over an attenuated live vaccine [8]. Paramount among these advantages is the elimination of the possibility of reversion to virulence of any attenuated live CAV vaccine. Up to now, irreversible attenuation of CAV is proving difficult because of the relatively simple genomic nature of this virus (13). This fact was first showed that after 100 passages of the CAV in cell cultures, pathogenicity was decreased, but was not completely lost (14). In addition, chicks derived from immune hens with virus neutralization (VN) antibody titers as low as 1:40 survived virus challenge and did not develop the disease, whereas it has also been suggested the VN antibodies titers of at least >8 log₂ (1:256) are necessary to prevent virus shedding in the feces and vertical transmission while the maximum present recorded titer was 1:128 (15).

Potency of the prepared live CAV vaccine showed detectable levels of specific CAV antibodies using SNT and ELISA by the 1st week post vaccination and reached peak titers by the 8th week post vaccination. In contact non-vaccinated birds attracted the excreted virus from vaccinated chickens and exhibited lower antibody titers on the 2nd week recording peak titer by the 12th week. Away control non-vaccinated chickens remain sero-negative allover 40 weeks. However, the recorded levels of antibodies began to decrease by the 36th week post vaccination although they remain high within group1 and 2. These results are tabulated in table (3 and 4). These results agree with findings which concluded that neutralizing antibody against CAV began to be detected 21 days PI in the chicks inoculated at 1 day of age, and 7 days PI in the chicks inoculated

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at 28 or 42 days of age(16). It has been recommended that CAV vaccination should guarantee a uniform development of high levels of VN antibodies in the breeder flocks to protect against vertical virus transmission and outbreaks of chicken infectious anemia in the progeny [17].

Regarding potency of the prepared inactivated CAV vaccine, detectable specific CAV antibodies were showed by 1st week post vaccination, reached the peak titer by the 8th week post vaccination then these titers began to decline by the 32nd week post vaccination. In contact and away control birds remain sero-negative all over the experimental revealing that there is no virus excretion. In this respect, It was said that prior to CAV vaccination at 20 weeks of age, all adult breeders chickens tested were negative for CAV antibodies, following vaccination with inactivated CAV vaccine at 20 weeks of age, antibody levels to CAV were detected in vaccinates at 30 weeks of age, and were maintained at relatively high levels in these birds until the final assay at 60 weeks of age [8]. Antibody level to CAV in vaccinates declined from 30 to 60 weeks of age. Antibodies to CAV were never detected in non-vaccinated birds for the duration of the experiment. A prepared inactivated CAV vaccine induced high levels of specific antibodies lasting for 24 weeks post vaccination [18] so, the use of inactivated CAV vaccine was recommended to avoid the disadvantages of the live attenuated vaccine which suppresses the immune response of chickens to other vaccines as fowl cholera and infectious coryza vaccines. In addition the use of an inactivated vaccine to CAV in adult breeding birds can provide effective protection in their progeny against disease following experimental challenge with virulent CAV [8]. The effectiveness of an inactivated vaccine is, to some extent, dependent on production of high titer virus. However, the use of suitable adjuvants in

combination with lower concentrations of virus can lead to development of highly immunogenic and stable vaccines. Depending on this fact montanoid oil ISA-70 was used as adjuvant to the inactivated vaccine which was known to act as immune modulator for poultry vaccines [19]. Generally, the inactivated CAV vaccine used in this study was clearly highly immunogenic, as demonstrated by substantial increases in CAV-SNT and ELISA reactivity following one application of the vaccine. These ELISA values declined slightly over the duration of the experiment. However, antibody levels to CAV were still detected by SNT and ELISA in vaccinated birds 40 weeks post-vaccination.

Hematocrit values evaluated in vaccinated chickens with CAV vaccine showed decreased levels by the 2nd week post vaccination in chickens vaccinated with live CAV vaccine, while chickens vaccinated with the inactivated CAV vaccine did not show decline in their hematocrit values that confirmed the complete safety of such vaccine. These findings come to be confirmed by those which concluded that protection against clinical disease in the progeny derived from breeders 40 weeks after vaccination was still effective as determined by hematocrit values and thymus weight [8]. They added that however, despite this, the global results indicate that the use of a CAV-inactivated oil adjuvant vaccine in the breeders protects most of the progeny from the disease.

In conclusion, the inactivated CAV vaccine used in this study was highly immunogenic, as demonstrated by substantial increases in CAV- SNT and ELISA reactivity following one application of the vaccine. In addition it did not show decline in the hematocrit values of vaccinated chickens that confirmed the complete safety of such vaccine..

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مجلة بنها للعلوم الطبية البيطرية



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المخلص العربي

يوجد مزارع كثيرة تستخدم لقاح أنيميا الطيور لتحسين الكناكيت ضد الإصابة بمرض انيميا الطيور، ولكن وجد أن اللقاح الحي يسبب المرض والهدف من هذا البحث هو تقييم ومقارنة امان وفاعلية اللقاح الحي والمثبط لأنيميا الطيور. استخدمنا لهذه التجربة 180 كتكوت خالية من المسببات المرضية (100 كتكوت تم اعطائهم اللقاح الحي و 50 كتكوت تم اعطائهم اللقاح الميت و 30 كتكوت كمجموعة حاكمة دون تحصين) وتم قياس المستوي المناعي دوريا على فترات حتى الاسبوع الرابع باستخدام اختبارات التعادل المصلى والأنزيم المرتبط المناعيا الممدص. أوضحت النتائج ان أطيور المحصنة باللقاح الحي عندها أجسام مناعية من الأسبوع الأول بعد التحصين ووصلت أعلى مستوى عند الأسبوع الثامن بعد التحصين. تبين أن الطيور الغير محصنة المتصلة مع الطيور المحصنة التقطت الفيروس من الطيور المحصنة باللقاح الحياوظهرت مستوى عيارية منخفضة لأجسام مناعية. أظهرت الطيور المحصنة باللقاح المثبط لانيميا الطيور أجسام مناعية من الأسبوع الأول بعد التحصين ووصلت أعلى مستوى فالأسبوع الثامن بعد التحصين وهذا المستوي بدأ يقل عند الاسبوع 32 بعد التحصين. الطيور الغير محصنة المتصلة مع الطيور المحصنة باللقاح المثبط لانيميا الطيور ليس لديها أجسام مناعية وكانت سالبة على مدار فترة التجربة. الطيور الغير محصنة والمنفصلة تماما و بعيدا عن الطيور المحصنة ليس لديها أجسام مناعية وكانت سالبة على مدار فترة التجربة. أظهر تقييم الهيماتوكريتفي الدجاج المحصن باللقاح الحي أن قيمة الهيماتوكريتقلت عند الاسبوع الثاني بعد التحصين وبدأ يرجع للطبيعى فى الاسبوع الثالث بعد التحصين وعلى الجانب الاخر الطيور المحصنة باللقاح المثبط والطيور الغير محصنة المتصلة مع الطيور المحصنة وتلكالغير محصنة المنفصلة والبعيدة تماما عن الطيور المحصنة قيمة الهيماتوكريت فيها طبيعى وهذا يؤكد أمان اللقاح المثبط عن اللقاح الحي. لقد استنتجنا فى هذه الدراسة أن لقاح انيميا الطيور المثبط كان أعلى مناعيا وأكثر أمانا من اللقاح الحي.

مجلة بنها للعلوم الطبية البيطرية: عدد 25 (2): 46-54, ديسمبر 2013