# Effect of vaccination with C*hicken anemia virus* vaccines on immune response to inactivated H5 vaccine

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## ABSTRACT

In this study two groups of nine week old Hubbard chickens were vaccinated with two different commercial live attenuated C*hicken anemia virus* (CAV) vaccines. One was vaccinated with 26 P4 strain vaccine subcutaneously and the other was vaccinated with Cux strain vaccine through drinking water (DW). Then the inactivated avian influenza (AI) H5N1 vaccine was inoculated intra-muscularly either at the 1st, 2nd or 3rd WPV with CAV vaccine forming three subgroups per each group. It is clearly observed that a relatively high levels of CAV ELISA antibody titers in all the vaccinated chicken subgroups at 4th WPV with either DW or S/C CAV vaccine indicating that the two CAV vaccines were immunogenic; Also, by measuring H5N1 HI titers within the 6 weeks post vaccination with AI vaccine in the sera of different chicken subgroups using HI test. It is clearly observed that AI HI antibody mean titers in S/C and in DW CAV vaccinated subgroups at the period between 3rd and 6th WPV with inactivated AI vaccine are high, homogenous and protective (titer range from $2^{7.8}$ to $2^{11}$) Also, these titers lay within the range of that titer of the vaccinated chicken with AI vaccine alone (AI control group) as it ranged between $2^{8.3}$ and $2^{10}$. Moreover, protective percentage of chickens in CAV subgroups post challenge with virulent H5N1 strain at 4th WPV with AI vaccine were satisfactory and equal to or above that of AI control group (80-85%). It is advisable to apply vaccination with the live attenuated CAV vaccines in poultry farms to select hygienically well controlled farms or even farms with closed system which maintain their birds in strong and well healthy state. Also it is preferable to vaccinate these birds with the inactivated AI vaccine at the 2nd WPV with either the DW or the S/C CAV vaccine. As the vaccinated birds showed the highest CAV ELISA Ab titers and the best immune response pattern to AI vaccine detected by HI test at this period.

## INTRODUCTION

Chicken infectious anemia (CIA) is a disease of young chickens caused by unique small virus circular DNA (Goryo *et al.*, 1987). Its infection causes anemia and severe immunosupression (Franz and Coral 2003) leading to problem with inadequate response to vaccination chicken anemia virus (CAV) was found to enhance the pathogenicity of a range of coinfection agents (Bulow *et al.* 1983). Immunosuppression causes by CAV thus causes serious economic losses (Nova *and Ragland.* 2001). Clinical disease of CAV is rare today because of wide spread of practice of vaccination in breeders with different types of CAV vaccines (Franz and Coral 2003). although (Pages Mante *et al.*, 1997) reported that live attenuated CAV have the possibility of reversion to virulence. Moreover Todd *et al.* (1998) reported that the irreversible attenuation of CAV is proving difficult. So these reports push us to study the safety of some commercial live attenuated CAV vaccines through monitoring the immune response to inactivated H5N1 AI vaccine in different groups of chickens which were vaccinated previously with different commercial CAV vaccines.

## MATERIAL

- **SPF embryonated chicken eggs (ECE)** were obtained from Koam Osheim SPF farm, Fayoum, Egypt and used in titration of virulent strain of H5N1 AI virus.
- **Experimental birds**: Two hundred hubbard breeder chickens were used for evaluation of the CAV vaccines.
- **Vaccines**
  - Nobilis CAV26P4 live attenuated CAV vaccine with a titer of 3.0 $\log_{10}$ $TCID_{50}$,/dose batch No. 116126R2, Intervet company.
  - Thymovac live attenuated CAV vaccine with a titer of 4.5 $\log_{10}$ $TCID_{50}$ 50/dose

CUX -1 strain, Lohmann Animal Health GmbH company.

- Inactivated H5N1 AI vaccine, oil emulsion vaccine contain inactivated reassortant AI strain A/ch/Egypt/A-18-H/2009.

- **Virulent virus strain for AI**:

Local high pathogenic ( HP) AI field isolates, identified as A/ch/Egypt /1709-6/08. Its titer was $10^{10}$ $EID_{50}$/bird. The titer was adjusted to be$10^5$ $EID_{50}$ /dose. and used as challenge virus for AI vaccines.

## METHODS

**- Experimental design**:

Two hundred breeder chickens free form antibody against CAV were used in this study. The birds were divided into three groups 1,2 and 3 ; 75 birds (group 1) were vaccinated with Thymovac live attenuated CAV vaccine through drinking water, 75 birds (group 2) were vaccinated with Nobilis CAV26 P4 live attenuated CAV vaccine subcutaneously (0.2 ml /bird) and 50 birds (group 3) were kept unvaccinated. group 1 and 2 were sub- divided into three subgroups (A, B and C) (25 chickens for each). Chicken in subgroup A, B and C were immunized with inactivated AI H5N1 vaccine intramuscularly (0.5 ml/bird) at 1st, 2nd and 3rd WPV with CAV vaccine respectively. Also, 25 chickens from group 3 were immunized with the inactivated AI H5N1 vaccine with the same rout and dose forming subgroup (3A) and act as AI control group while the rest 25 chickens in group 3 left as negative control neither vaccinated with CAV nor with H5N1 AI vaccine forming subgroup (3B). Blood samples were collected from different subgroups (ten birds/subgroup) weekly post vaccination with H5N1AI vaccine beginning from the second WPV with AI vaccine till 6th WPV. Blood sera were prepared and inactivated at 56 ºC for 30minute and kept at -20ºC.

**Challenge test**

According to (Egyptian Standard Regulation, 2009) chickens from each subgroup were challenged with 0.1 ml of HPAI ($10^5$ $ED_{50}$/bird) intramuscularly at 4th WPV with AI vaccine. All challenged chickens were observed daily for 10 days. The mortality and morbidity rates were recorded to measure protection percentage.

**Serological tests:**

- ELISA test using ELISA kit for detection of CAV ELISA antibody, Synbiotics Corporation SAN DIEGO, CAq 2127, U.S. Vet lic No. 312. The kit was used according to the manufacturer instruction.

- **Haemagglutination inhibition (HI) assay**: was done according to the standard protocol of OIE (2013) to detect AI antibody titers against AI virus using inactivated AI homologous antigen A/ch/Egypt/A-18-H/2009 strain and standard antisera against the same AI strain using 1 % chicken RBCs.

## RESULTS

It is clearly observed in Table (1) that CAV ELISA mean titers in all vaccinated groups with either drinking water CAV vaccine (sub group 1 A, 1 B and 1 C) or with S/C CAV vaccine (subgroup 2A, 2B and 2 C) showed highly increase CAV ELISA mean titers (ranged between 5288 and 8509) at 5th WPV with CAV vaccine if compared with control negative unvaccinated subgroup 3B (1782.5). Also, CAV mean titers in chicken subgroups vaccinated with Cux strain vaccine through DW were higher than that of subgroups vaccinated with 26 P4 strain vaccine through S/C route specially in subgroup (B and C ) as CAV ELISA titers in DW groups ranged between 5908 and 8509 while that of S/C subgroups ranged between 5288.6 and 6801 at the same period post vaccination with CAV. In addition, it is observed that subgroup 1 B and 2 B which were vaccinated with AI vaccine at 2nd WPV

with either DW or S/C CAV vaccine respectively showed the highest level of CAV ELISA mean titers if compared with that of groups which were vaccinated with AI vaccine at 1st or 3rd WPV with CAV vaccine (subgroup 1A, 1C, 2A and 2C).

It is clearly observed that AI HI antibody titres in the sera of chickens in CAV vaccinated subgroups as well as in AI control subgroup were highly increased at the third WPV with AI vaccine if compared with that titers at the 2nd WPV and at the pre-vaccination period then the titers steady increased at the period between the third and sixth WPV. These high homogenous AI HI titers ranged between 7.8 and 11 and considered response to the inactivated AI vaccine (Table 2). On the other hand, a low percentage of chickens (10 to 30 %) in some CAV vaccinated subgroups showing negative AI HI titers at the 3rd and 4th WPV with AI vaccine in case of most of S/C CAV vaccinated subgroups and at 6th WPV in case of D.W. CAV vaccinated subgroups which were vaccinated with AI vaccine either at 1st or at 3rd WPV with CAV vaccine. The best time of vaccination with H5N1 vaccine was observed at the second WPV with CAV in case of S/C vaccination where there is no negative AI HI titers while in case of vaccination by D.W. route, the best time also at 2nd WPV with CAV where the negative titers observed with low percentage (10%) at 6th WPV.

The protection percentage of chicken in CAV vaccinated subgroups post challenge with virulent AI virus at 4th WPV with inactivated AI vaccine were satisfactory and equal to or above that of the AI control subgroup as they ranged between 80-85%. These results are parallel to that of the immune response to AI vaccine pattern which were detected by the HI test as the AI HI antibody titers were satisfactory at the period between 3rd and 6th WPV with AI vaccine.

**Table (1):** Mean antibody titers for chicken groups at 5th WPV with CAV vaccine using ELISA

| **Strain and route of CAV vaccination** | | **Subgroup (Time of vaccination with AI vaccine)** | **Mean CAV ELISA antibody titers** |
|---|---|---|---|
| Chicken Groups | (Group 1) Cux strain through drinking water | 1A At 1st WPV with CAV vaccine | 5908 |
| | | 1B At 2nd WPV with CAV vaccine | 8509 |
| | | 1C At 3rd WPV with CAV vaccine | 8121 |
| | (Group 2) 26 P4 strain through S/C route | 2A At 1st WPV with CAV vaccine | 5456 |
| | | 2B At 2nd WPV with CAV vaccine | 6801 |
| | | 2C At 3rd WPV with CAV vaccine | 5288.6 |
| | (Group3) CAV unvaccinated | 3B | 1782.5 |

**Table (2):** Mean antibody titers in chicken groups at different period post vaccination with inactivated AI vaccine using HI assay expressed as $log_2$

| Strain and route of CAV vaccination | Subgroup | Time of H5N1 vaccination | Mean AI HI titers expressed by log 2(% of negative birds)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Weeks post vaccination with inactivated AI vaccine | | | | | | | | | |
| | | | Pre vaccination | 2 | 3 | | 4 | | 5 | | 6 | |
| | | | | | * +ve | ** -ve | +ve | -ve | +ve | -ve | +ve | -ve |
| (Group 1) Cux strain through drinking water | 1 A | 1st WPV with CAV vaccine | 0 | 2.3 | 100% 7.8 | - | 100% 9 | - | 100% 10 | - | 70% 10.1 | 30% 0 |
| | 1B | 2nd WPV with CAV vaccine | 0 | 3.1 | 100% 8.8 | - | 100% 9.3 | - | 100% 9.8 | - | 90% 11 | 10% 0 |
| | 1C | 3rd WPV with CAV vaccine | 0 | 6 | 100% 9 | - | 100% 9.7 | - | 100% 10 | - | 80% 10.3 | 20% 0 |
| (Group 2) 26 P4 strain through S/C route | 2A | 1st WPV with CAV vaccine | 0 | 1.5 | 80 % 8.3 | 20 % 0 | 83% 9.3 | 17% 0 | 100% 10.5 | - | 100% 10.6 | - |
| | 2B | 2nd WPV with CAV vaccine | 0 | 4.8 | 100% 8.6 | - | 100% 9 | - | 100% 9.5 | - | 100% 10 | - |
| | 2C | 3rd WPV with CAV vaccine | 0 | 5 | 78% 8.5 | 22% 0 | 90% 9 | 10 % 0 | 100% 9 | - | 87% 9.6 | 13 % 0 |
| (Group 3) CAV unvaccinated | 3A | H5N1 vaccinated | 0 | 3 | 100% 8.3 | - | 100% 9.1 | - | 100% 10 | - | 100% 10 | - |
| | 3B | H5N1 unvaccinated | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Percentage of chickens showing positive AI HI titers.
** Percentage of chickens showing negative AI HI titers.

**Fig. (1): Mean AI HI antibody titres expressed as $log_2$ in chicken subgroups at different period post vaccination with inactivated AI vaccine using HI assay**

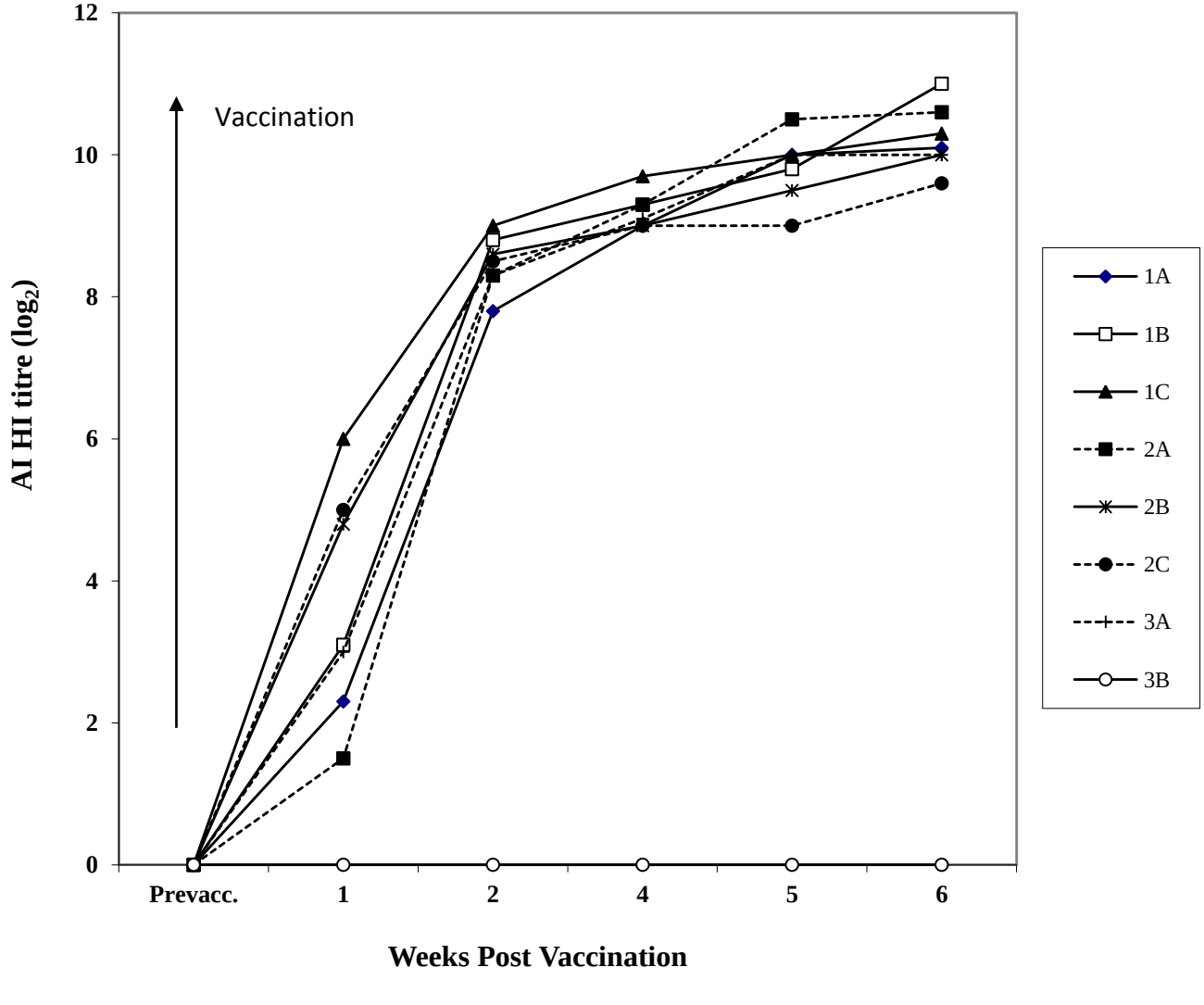

**Table (3):** Protection percentage in chicken subgroups post challenge with virulent H5N1 strain at 4th WPV with inactivated H5N1 AI vaccine and their relation to $log_2$ of AI HI antibody titers at different time of challenge.

| Strain and rout of CAV vaccination | Chicken Subgroup | Time of AI vaccination | No Of Birds | No. of Dead Birds (Days post challenge) | | | | | | | | | | Alive No /total | Protection percentage | AI HI titers at time of challenge |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
| (Group1) Cux strain through drinking water | 1 A | 1st WPV with CAV vaccine | 15 | 2* | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 11/13 | 84 | 9 |
| | 1B | 2nd WPV with CAV vaccine | 15 | 1* | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12/14 | 85 | 9.3 |
| | 1C | 3rd WPV with CAV vaccine | 15 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 12/15 | 80 | 9.7 |
| (Group 2) 26 P4 strain through S/C rout | 2A | 1st WPV with CAV vaccine | 15 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 12/15 | 80 | 9.3 |
| | 2B | 2nd WPV with CAV vaccine | 15 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 12/15 | 80 | 9 |
| | 2C | 3rd WPV with CAV vaccine | 15 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 12/15 | 80 | 9 |
| (Group 3) CAV unvaccinated | 3A | AI vaccinated | 15 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 12/15 | 80 | 9.1 |
| | 3B | AI unvaccinated | 15 | 0 | 0 | 12 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 | 0 | 0 |

* Deaths at first day post challenge are considered non-specific

## DISCUSSION

The DW or S/C CAV vaccine application resulted in relatively high levels of CAV ELISA antibodies in the vaccinated chicken subgroups at the 5th WPV with CAV vaccine compared with that of the control unvaccinated chicken subgroups. These results come in accordance with other research conducted on the use of CAV vaccines (Pages-Mante *et al.*, 1997, Brentanol *et al.* 2005 and Hanan *et al.*, 2008). In addition, Most chickens in subgroups which were vaccinated with inactivated AI vaccine at 1st, 2nd and 3rd WPV with either DW or S/C CAV vaccine responded well to the AI vaccine as the HI titer patterns developed normally and nearly resemble that of the AI vaccinated control subgroup .Moreover all the H5N1 AI HI antibody titers expressed as $\log_2$ at the third WPV with AI vaccine on word are protective (>7) according to Egyptian Standard Regulation (2009) as shown in Table (2) and Figure (1). This result comes in agreement with the result of challenge test as the protection percentages in all CAV vaccinated chicken subgroups post challenge with virulent H5N1 AI strain at the 4th WPV with AI vaccine were satisfactory and ranged between 80-85% according to the Egyptian Standard Regulation (2009). These values are equal to or above that of the AI control subgroup as shown in Table (3). Consequently it is concluded that vaccination with either DW or S/C CAV vaccine one or two or three weeks before immunization with inactivated AI vaccine didn't cause any immune suppression on most of the vaccinated birds as the immune response to AI vaccine developed normally in most birds of all S/C and DW CAV subgroups. These results agreed with Hanan *et al.* (2008) but it is clearly observed that there are low percentage of birds in subgroups which vaccinated with CAV vaccine one week or three weeks before immunization with AI vaccine didn't respond to the AI vaccine in case of most of CAV vaccinated subgroups. These low percentages of birds which not responded to AI vaccine may have any subclinical disease at time of vaccination as they were reared in open system place which may cause immune suppression to the birds. All these factors make these low percentage of birds negatively affected by the vaccination with the live attenuated CAV vaccines at 3rd and 4th WPV with AI vaccine in case of most of S/C CAV vaccinated subgroups and at later period (6th WPV) in case of D.W. CAV vaccinated subgroups. The difference in time of occurrence may be due to the difference in route of immunization (Tan and Tannock, 2005) between S/C and D.W CAV subgroups.

In conclusion, it is preferable to vaccinate strong healthy birds with live attenuated CAV vaccines and to maintain their health along the breeding period as the live attenuated CAV vaccinal strain negatively affect that weak subclinically diseased birds as the pathogenicity of the Cux or 26 P4 vaccinal strains which were attenuated on the cell culture decreased but not completely lost (Bulow and Fuchs, 1986). In addition it is preferable to vaccinate birds with the inactivated AI vaccine at the second WPV with the live attenuated CAV vaccines as the vaccinated birds at this period showed the highest CAV ELISA antibody titers and the best immune response pattern to AI vaccine if compared with 1st and 3rd WPV.

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