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Serological and Molecular Testing of Chicken Infectious Anemia Virus in Vaccinated Chicks with Commercial CuxStrain

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Abstract

Chicken infectious anemia virus (CIAV) has been placed in the list of emerging viruses that cause severe threat to the Egypt poultry industry. Enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were applied for testing CIAV in vaccinated chicks in two experiments. In experiment (1) chicks at 1-day old were subcutaneously vaccinated with original stock or diluted preparations (from 10^{-1} to 10^{-6}) from commercial CIAV-vaccinal strain (Cux strain). In experiment (2) chicks at 7-day old were vaccinated with experimentally contaminated live NDV vaccine with CIAV Cux strain by eye drop (G2) parallel to that vaccinated with CIAV subcutaneously (G1) and other control non-vaccinated group (G3). ELISA detected CIAV antibodies in sera of vaccinated chicks with original stock or diluents preparations (till 10^{-3}) from Cux strain at 21day post-vaccination (dpv) while CIAV DNA was detected in thymus, spleen and liver in most of the vaccinated chicks with original stock or diluents preparations (till 10^{-6}) at 14 dpv by PCR. For experiment (2) ELISA could detect CIAV antibodies in sera by the second week post-vaccination in G1 and by the third week post-vaccination in G2 while PCR able to detect CAV DNA in liver and spleen tissues by the second week post-vaccination in G1 and G2. In conclusion, the commercial CIAV- Cux strain induce humoral response associated with viral persistency in spleen, thymus and liver in vaccinated chicks limit its usage in young chicks. PCR and ELISA were tests of choice for current testing CIAV in vaccinated chicks.

Keywords: CIAV, ELISA , PCR, Vaccinated Chicks.

1. Introduction

Chicken infectious anemia virus (CIAV) is a Gyrovirus belonging to the family Circoviridae. The agent is a small (approximately 20–25 nm in diameter), non-enveloped, spherical in outline with icosahedral symmetry [17]. The viral genome consists of a circular, negative-sense, single stranded DNA of 2.3 kb that contains three partially overlapping major open reading frames (ORFs) encoding three proteins; namely, VP1, VP2 and VP3 [13].

The VP1 (51 kD) acts as a major capsid protein, VP2 (24 kD) acts as a scaffold protein and is essential for the virus assembly, while VP3 (13 kD), also known as apoptin, is important for the disease pathogenesis and apoptosis [14] Both VP1 and VP2 are required to induce protective immunity [17] . In Egypt, CIAV has been suspected since long on the basis of clinical symptoms and post mortem lesions in the major poultry raising states of the country [12,2] .

The role of CAV as a vaccine contaminant is well established. The virus usually introduces to the avian vaccines prepared in embryonating eggs, which get the infection vertically [11].

Therefore, the process of assuring freedom of the avian virus vaccines from CAV contamination requires a 2-step strategy; first, testing the SPF embryonating eggs used in vaccine production and quality control; and second, examining the produced vaccines for possible contamination before release. Current flock testing for CIAV infection or vaccination is usually based on the presence of antibodies detected by enzyme-linked immunosorbent assay (ELISA). However, the presence of antibodies can no longer be considered to be an exact indication of the presence or absence of CIAV [3]. Sometimes DNA testing is necessary to assess the CIAV status of birds [11,5]Polymerase chain reaction (PCR) assays have become the assay of choice for the detection of ClAV DNA in infected cell cultures, chicken tissues, archived formalin-fixed paraffin-embedded tissues, or vaccines [18].

The test proved to be specific and definitely more sensitive than cell-culture isolation of the virus [19]. Because of the potential role of immunosuppression due to CIAV infection and decreased responsiveness to vaccines against other viral agents, we determined the ability of PCR for testing the presence of CIAV DNA in experimentally contaminated viral vaccines as well as comparing ELISA and PCR for testing CIAV in vaccinated chicks.

2. Material and methods

2.1 Commercial CIAV-vaccinal strain (Cux strain)

The European attenuated Cux strain of CIAV on MSBI cells with a titer of $10^{5.5}$ $TCID_{50}$/vial were kindly obtained from veterinary serum and vaccines research institute (VSVRI), Abbasia, Cairo. The vaccine was supplied with a trade name Aviprothymo-vac by LAH Company (batch no. A041511) and was sufficient for 1000 doses. This Commercial CIAV-vaccinal strain was used in the experiments of this study.

2.2 Chicks and experimental design

Experiment (1): One hundred, and ten (110) one day old SPF chicks were used in this experiment. Chicks were divided into eight groups from I to VIII each group contains 14 chick except for the control unvaccinated (VIII) contain 12 chick. They reared separate in isolators. The Commercial CIAV vaccine vial containing 1000 doses in 10 ml was diluted in 10-fold series from 10^{-1} - 10^{-6}. The original vaccine stock and the six dilutions were used for vaccination of one chick group where each chick at 1 day old received one dose (0.1 ml) by S/C route**.** The sera from chicks were collected 21 day post vaccination for ELISA testing CIAV antibodies. Also, the spleen, thymus and liver were taken 14 day post vaccination for PCR testing CIAV DNA**.** Experiment (2): One hundred, and fifteen (115) 7 day old SPF chicks were used in this experiment. Chicks were allotted into G1, G2 and G3 groups and reared separate in isolators. The divided groups were as follow**:** G1: each of 30 chicks was vaccinated subcutaneously with 0.1 ml of the commercial CIAV-vaccinal strain (Cux strain) as single dose. G2: each of 35 chicks was vaccinated by one eye drop with contaminated live NDV (clone 30) vaccine with Cux strain (1:1) as single dose. G3: the 50 chicks left as non-vaccinated group. The sera and tissue (liver and spleen) samples from chicks were collected till 5th week post vaccination for testing CIAV antibodies and DNA using ELISA and PCR respectively.

2.3 Serum samples

The sera collected from chicks in experiment (1) and (2) were inactivated at 56°C for 30 minutes, and then stored at -20°C till used in ELISA.

2.4 Tissue samples

Pools of liver, spleen and thymus were collected from different chick groups at 14 days Post vaccination. Samples of each group were pooled as one sample at a time. Samples of uninoculated chicks were served as negative controls parallel to test samples. The collected organs were washed in sterile 0.85% saline, and frozen at below-10°C. After thawing, the tissue homogenates (10% W/V) were prepared in sterile saline 0.85% containing 1000 IU/mL penicillin, 1.0 mg/ml streptomycin. By disrupting organs using sterile mortar and pestle, the homogenates were then centrifuged at 3000 rpm for 20 min. then DNA was extracted and utilized for detection of CAV by PCR**.**

2.5 Enzyme linked immunosorbent assay (ELISA)

Synbiotics commercial ELISA kit was used for detection of CIAV antibodies in sera of vaccinated chicks. It was carried out according to the manufacturer instruction.

2.6 Polymerase chain reaction (PCR)

The reaction was performed as following condition according to [15] using VP1gene primer obtained from Bio Basic Canada Inc. The expected product size is 676 bp. The sequences of primer used were as follow sense Sequence (5`to`3): GACTGTAAGATGGCAAGACGAGCT anti sense Sequence (5`to`3): GGCTGAAGGATCCCTCATTC **.**

3. Results

The Synbiotics commercial ELISA kit detected CIAV antibodies in sera of vaccinated 1 day old chicks with original stock or diluents preparations (till 10^{-3}) from commercial CIAV-vaccinal strain (Cux strain) at 21day post-vaccination table (1,2) while CIAV DNA was detected in thymus, spleen and liver in most of the vaccinated 1 day old chicks with original stock or diluents preparations (till 10^{-6}) at 14 dpv by PCR fig (1) and table (2). ELISA and PCR testing of CIAV in vaccinated chicks with experimentally contaminated live NDV vaccine with CIAV Cux strain (G2) parallel to that vaccinated with CIAV (G1) for successive five week post-vaccination.it was found that ELISA could detect CAV antibodies in sera by the second week post-vaccination in G1and by the third week post-vaccination in G2 table (3) while PCR able to detect CAV DNA in liver and spleen tissues by the second week post-vaccination in G1 and G2 table (3) and fig (2).

Table (1) Results of ELISA for testing CIAV antibodies in sera of vaccinated chicks with different preparation of CIAV (Cux strain) vaccine at 21th day post-vaccination

Chick group	Vaccination	Number of tested bird	Mean s/p ratio ± Standard deviation	Suspicion
I	From original stock	8	0.71 ± 0.18	Positive
II	From dilution 10^{-1}	8	0.47 ± 0.05	Positive
III	From dilution 10^{-2}	8	0.66 ± 0.34	Positive
IV	From dilution 10^{-3}	8	0.66 ± 0.08	Positive
V	From dilution 10^{-4}	8	0.35 ± 0.006	Negative
VI	From dilution 10^{-5}	8	0.19 ± 0.001	Negative
VII	From dilution 10^{-6}	8	0.14 ± 0.001	Negative
VIII	Unvaccinated (control)	6	0.34 ± 0.01	Negative

S/P ratio less than or equal to 0.35 is - ve result, S/P ratio more than 0.35 is + ve result.

Table (2) Comparative results of testing CIAV in vaccinated chicks with different preparation of CIAV (Cux strain) vaccine by ELISA and PCR

Chick group	Vaccination	Testing CIAV	
		ELISA* (21^{th} day post-vaccination)	PCR** (14^{th} day post-vaccination)
I	From original stock	+	+
II	From dilution 10^{-1}	+	+
III	From dilution 10^{-2}	+	+
IV	From dilution 10^{-3}	+	+
V	From dilution 10^{-4}	-	+
VI	From dilution 10^{-5}	-	+
VII	From dilution 10^{-6}	-	+
VIII	Unvaccinated (control)	-	-

* Detection of CAV antibodies in chick sera, **detection of CAV DNA in liver, spleen and thymic tissues

Table (3) Comparative results of testing CIAV in vaccinated chicks with experimentally contaminated live NDV vaccine with CIAV (Cux strain) parallel to other groups by ELISA and PCR

Wpv*	G1		G2		G4	
	ELISA	PCR	ELISA	PCR	ELISA	PCR
1st week	-	-	-	-	-	-
2nd week	+	+	-	+	-	-
3rd week	+	+	+	+	-	-
4th week	+	ND	+	ND	-	ND
5th week	+	ND	+	ND	-	ND

4. Discussion

Testing CIAV in vaccinated chicks with original stock or diluents preparations (till 10^{-6}) from commercial CIAV-vaccinal strain (Cux strain) demonstrate that the vaccine induce humoral response associated with viral persistency in spleen, thymus and liver in 1-day old chicks. As chickens get older, they rapidly develop a resistance to the experimentally induced disease although they remain susceptible to infection. This resistance is virtually complete by the time the birds reach 2 weeks of age[1]. The attenuated CIAV strains are commercially used as vaccine but some technical and practical problems affect the widespread use of vaccines [16] Antibody responses are the major arm of protective immunity to ClAV, but neutralizing antibodies cannot be detected until 3 weeks PI of susceptible one-day-old chicks [4].

The present study reveals that the original stocks and diluents preparations till 10^{-3} from commercial attenuated CIAV strain induce humoral immune response that detected by ELISA at 21day post-vaccination and failed to induce by other preparation of vaccine. [found that higher virus levels corresponded to higher antibody levels detectable by ELISA, suggesting that higher antibody levels were a result of greater stimulation by virus. Positive results indicate an earlier CIAV infection with sufficient production of viral proteins to elicit an antibody response [20]. However, on the basis of studies by [3] the presence of antibodies can no longer be considered a true indication of the presence or absence of the virus.

Replication of the vaccinal viral strain tested in the present work was detected in thymus, spleen and liver in most of the vaccinated chicks, at 14 dpv by PCR original stock or diluents preparations (till 10^{-6}) from commercial CIAV-vaccinal strain (Cux strain). This indicates viral un-clearance by this organ. It is demonstrated that pathogenic CIAV was produced in many organs, both in 1 day old or 6 week-old infected chicks, up to day 18 post-inoculations and reached a peak in the thymus, spleen and liver at 18 or 20 days pi [9]. The vaccinal strain used in our work despite their attenuation since viral VP1 gene became detectable after 14 dpv, suggesting a viral persistency in this organ. [9] showed that persistent viremia occurs in CIAV infected birds in the absence of antibody production. The viral persistency of pathogenic CIAV was confirmed by [7]who suggested that CIAV can induce persistent infection in infected birds. These observations are comparable to a previous finding in which some attenuated CIAV strain appear in spleen and thymus at the same time following infection at 1 day of age [1,10].

The virus persistence and the immune disorders induced by the CIAV vaccine virus lead to practical consequences, and it is likely to play an important role in the sub-clinical infections and decreased responsiveness to other avian pathogens in the poultry industry

In our study, incompetent immune system of the very young chicks, decrease of the CD4+ cells in the thymus and spleen, decrease in percentage of IgM-bearing cells and persistency of vaccinal strain in thymus and spleen might result in inability of the

inoculated birds to produce proper high levels of anti-CIAV antibodies. Presence of the virus genome in the spleen coexisted with lack of functional humoral immunity in the inoculated birds. It was previously reported that pathogenic CIAV was recovered from blood cells or lymphoid organs of infected birds at different days pi even in the presence of low or high viral neutralizing antibodies [21,7].

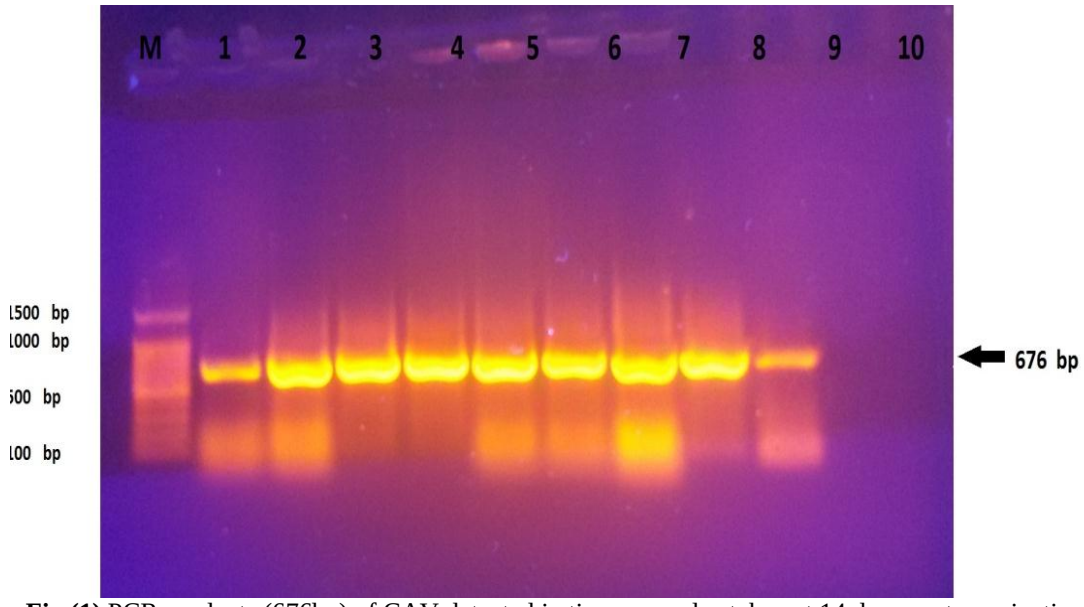

Fig (1) PCR products (676bp) of CAV detected in tissue samples taken at 14 days post-vaccination with ten-fold dilutions of CAV (Cux strain) vaccine. Lane 1, 8, 9: tissues of chicks vaccinated with original vaccine stock. Lanes 2-7: tissues of chicks vaccinated with 10-fold serial dilutions from 10^{-1} to 10^{-6} of Cux strain of CAV vaccine respectively. Lane 10: Negative control

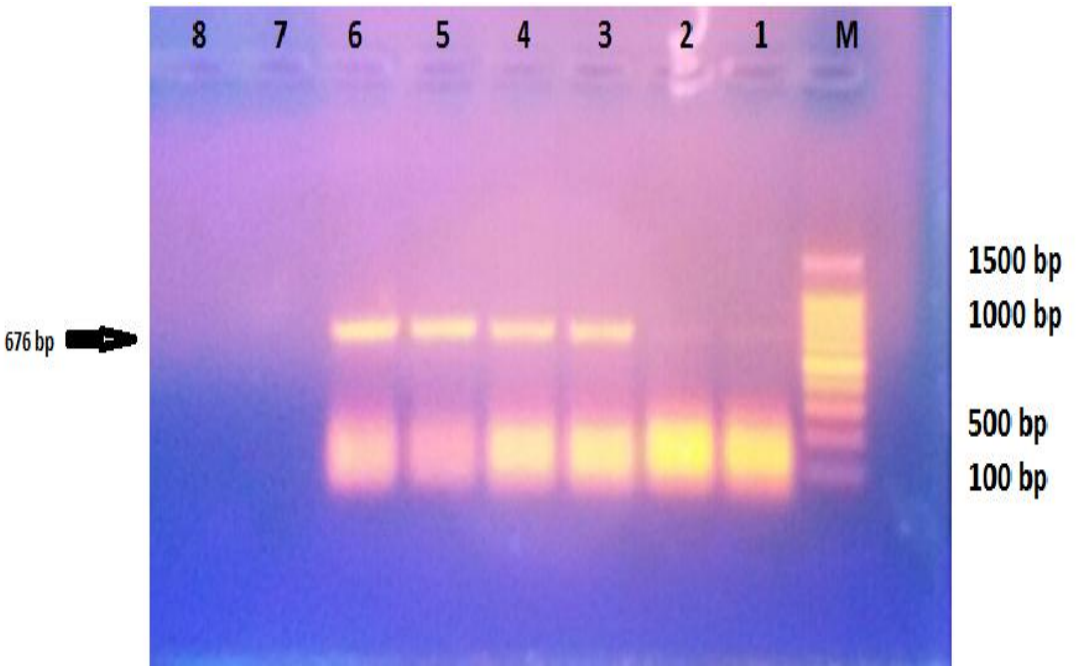

Fig (2) PCR products (676bp) of CAV detected in tissue samples (liver and spleen) of chicks vaccinated with experimentally contaminated live NDV vaccine with CIAV (Cux strain)on the 1st, 2nd, 3rd weeks post-vaccination lanes 2, 4, 6 while lane 1, 3, 5: tissues (liver and spleen) of chicks vaccinated with CIAV (Cux strain) on the1st, 2nd, 3rd weeks post-vaccination. Lane 10: Negative control.

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