

The Negative Impact of Chicken Infectious Anemia Virus Infection on Immune Responses to Different Newcastle Disease Virus Vaccination Programs

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Abstract

Chicken infectious anemia virus (CIAV) is among the naturally occurring viruses that cause immunosuppression in chickens. In the present study, the impact of CIAV infection on both NDV vaccination immune responses and bird performance were demonstrated. Post CIAV infection, the mean live body weights of the ND-vaccinated groups showed improvement compared with the non-NDV vaccinated groups. The immunosuppressive effects of CIAV infection was higher and more obvious on live ND vaccinated group compared with the other vaccinated groups. The vaccination with combination of live and inactivated vaccines showed better HI-NDV Ab responses especially when inactivated vaccine was administered at 9 days (group II). In consistent, both the phagocytic Percent and Index were lower in case of vaccination with live NDV vaccine only and CIAV infection. Birds were vaccinated with both live and inactivated NDV vaccines at different time points showed superior protection rate (100%) compared with the other groups. Microscopically, CIAV infection led to severe deterioration in the lymphoid tissues (thymus, bursa, spleen and bone marrow) such as focal to diffuse depletion of lymphocytes and hematopoietic cells. This study highlights the negative impact of CIAV infection on both bird performance and immune status.

Keywords: Chicken, ND, Anemia and Histopathology, Vaccine

Introduction

Chicken infectious anemia virus (CIAV) is one of the extensively studied viral pathogens of poultry and has gained much attention due to its emerging and reemerging nature [1,2]. It leads to major economic losses in the poultry industry due to its severe immunosuppressive potential, vaccination failure, production losses and increased mortality associated with multiple secondary infections [3,4]. The virus was first isolated in 1979 in Japan [5]. It is a member of *Gyrovirus* genus of the family *Circoviridae* [6], non-enveloped, has a small diameter (22 nm), icosahedral symmetry and circular DNA genome [7]. Chicken is the only recognized host of the virus, and the disease generally occurs in young chicks of up to 3-4 weeks of age with no maternal antibodies, especially at one day-old [8]. This disease is characterized by increased mortality, poor weight gain, reduction in haematocrit values, anaemia, development of subcutaneous and intramuscular hemorrhage, aplasia of the bone

marrow and generalized lymphoid atrophy [9-12].

Chickens older than 2-3 weeks of age are also susceptible to infection but only develop a subclinical disease that can result in immunosuppressive effect [10,13]. The disease has a potent immunosuppressive effect of both cellular and humoral immune responses [14]. Therefore, it can cause depression of immune response against several virus vaccines such as NDV, MDV and infectious laryngotracheitis (ILT) leading to vaccination failure [8,15].

Despite the intensive vaccination programs, the Egyptian poultry industry faces a major threat of velogenic viscerotropic NDV (vvNDV) outbreak, up till now the definite reason for these outbreaks is not obvious. This study aimed to evaluate the role of one of the most important immunosuppressive diseases, CIAV, in the vaccination failure phenomena against NDV.

Material and Methods

Birds and housing

Sixty, one day-old, SASSO broiler chicks were obtained from Alwafaa-SASSO Breed Company, Giza, Egypt, and were divided into five equal groups. The chicks were housed under standard environmental and hygienic conditions. The starting temperature was 33°C, then decreased gradually 2°C each week until reached 20°C at the end of 8th week with continuous lighting throughout the experiment. The chicks were fed on starter ration with (23%) protein obtained from Cairo Poultry Company (CPC), Egypt, throughout the experimental period.

Viruses

Sheble and Reda, 1976 vvNDV ($10^{6.8}$ EID₅₀/mL) and CIAV were kindly obtained from Dr. Samir A. Nassif, Chief Researcher of Poultry Diseases and Head of Biotechnology Department, Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abbasia, Cairo.

Vaccines

Newcastle disease vaccines including ND HitchnerB1 vaccine (live attenuated NDV strain, containing at least $10^{6.5}$ EID₅₀/Izo S.U.R.L. (Italy), Batch no. 02461), ND LaSota vaccine (live attenuated vaccine containing at least 10^6 EID₅₀, Intervet UK Ltd, Batch no. 14603IJ01) and Newcavac vaccine (inactivated ND Clone 30 virus, Intervet UK Ltd, Batch no. S257A01) were used in the study.

Experimental design

Sixty, one day-old, SASSO broiler chicks were allocated into five experimental groups (I, II, III, IV and V), each group contained 12 chicks. All birds of groups I, II, III and IV were infected with CIAV strain at day one of age with a dose of 0.2 mL via intramuscular (IM) injection. Groups I, II and III received different NDV vaccination programs, birds of group I were vaccinated with HitchnerB1 and LaSota vaccines at 7 and 18 days of age, respectively, via eye instillation. While, birds

of group II were vaccinated with Hitchner B1, inactivated oil emulsion and LaSota vaccines at 7, 9 and 18 days of age via eye instillation, S.C injection and eye instillation, respectively. Birds of group III were vaccinated with Hitchner B1, inactivated oil emulsion and LaSota vaccines at 5,7 and 18 days of age via eye instillation, S.C injection and eye instillation respectively. Group IV (CIAV-infected non NDV-vaccinated) and group V (non CIAV-infected non NDV-vaccinated) kept as control groups.

At 39 days of age (21 days post last vaccination), groups I, II, III and IV were challenged with very vvNDV strain (0.5 mL IM/bird) at a dose of 10^6 EID₅₀/bird. Group V was subdivided into 2 subgroups (A and B), subgroup A remained as a negative control and subgroup B was challenged with vvNDV strain 0.5 ml I/M/bird ($10^{6.8}$ EID₅₀/ml).

Birds were bled at 7, 14, 21 and 28 days old on heparin anticoagulant for phagocytic activity test. Sera samples were obtained weekly for 8 weeks for serological tests.

Specimens were collected from thymus, bursa of fabricius, spleen, liver and bone marrow 6 days post CIAV infection at 7, 14, 21 and 28 days-old in order to perform histopathological examination. Lungs, liver, proventriculus and intestine were collected from the challenged chickens with vvNDV two days post challenge in order to examine histopathological changes caused by the virus infection. The tissues were fixed in 10% buffered neutral formalin solution for histopathological sections.

Haemagglutination inhibition test (HI test)

It was performed as previously mentioned [16]. Briefly, the micro HI test was performed using 10 HA units of the LaSota vaccine strain of NDV and 1% chicken red blood cells. Serum samples were pre-treated at 56°C for 30 min to inactivate the nonspecific agglutinin then diluted into 1/5 and a duplicate two-fold dilution series of each tested serum. Titers were expressed as log values of the highest reciprocal of the dilution which showed hemagglutination inhibition.

Phagocytic activity test

Separation of peripheral blood mononuclear cells, phagocytic assay and evaluation of phagocytic activity were performed according to Goddeeris *et al.* [17].

Histopathological examination

Collected specimens were fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by H&E and then examined microscopically [18].

Statistical analysis

Data were analyzed using repeated measures ANOVA analysis of variance (ANOVA) and General Linear Models (GLM) using the Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., Chicago, IL, USA). The comparison of means was carried out with Tukey's Honestly Significant Difference (Tukey's

HSD) test. Results are presented as mean \pm standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance.

Results

Clinical findings

In case of CIAV-infection, birds showed dullness, depression, diarrhea, ruffled feathers and impaired growth fourteen days post infection (PI). The signs were clear and more evident in case of absence of NDV vaccination (group IV) in comparison with chicks of groups I, II and III. The experimental birds of group V (negative control) had no observed clinical signs.

The gross findings of the experimentally CIAV-infected birds, 14 days PI, revealed paleness of breast muscles, icteric liver, atrophy of the thymus loops, pink bone marrow and hemorrhage on the breast muscles (Figure 1).

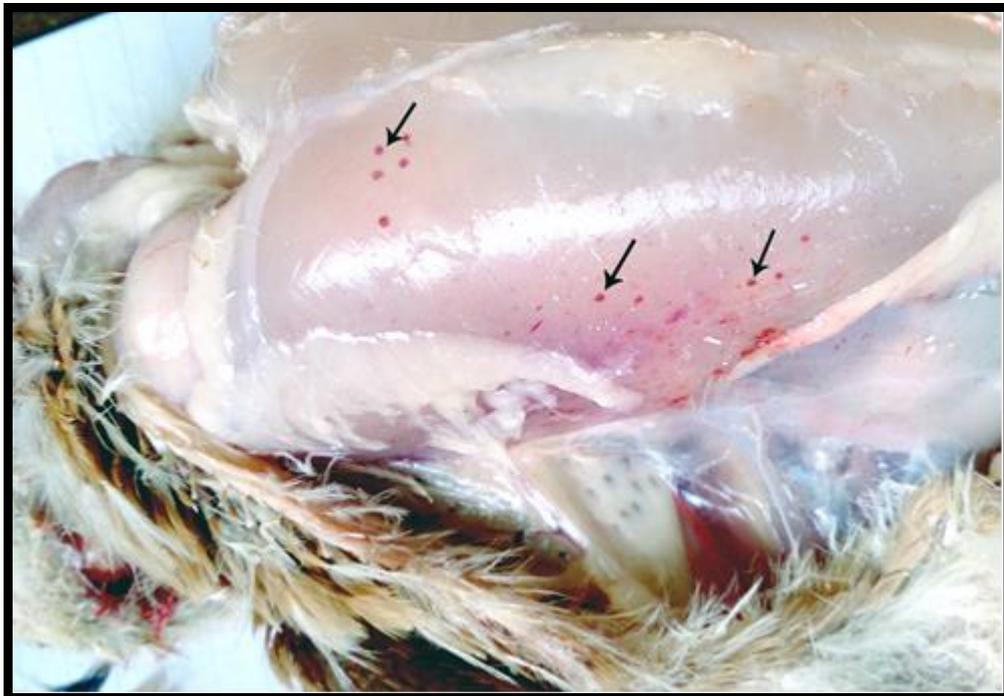


Figure (1): Gross pathology of CIAV infected birds. Chickens of group IV experimentally infected with CIAV strain only showing hemorrhage on the breast muscles.

Body weight

As shown in Table 1, the live mean body weight in case of CIAV infection and NDV vaccination (groups I, II & III) revealed a significant decrease in the live mean body

weight compared to the negative control group (group V). On the other hand, the same groups showed significant increase in the live mean body weight compared to the CIAV infected non NDV vaccinated group (group IV).

Table 1: Effect of CIAV infection on the live mean body weight of different NDV vaccination programs

Group	1-day	W 1	W 2	W3	W4	W5	W6	W7	W8
1	34.17 ±0.56 ^a	96.25 ±3.60 ^b	245.56 ±11.04 ^b	446.25 ±12.34 ^b	659.29 ±13.13 ^c	936.43 ±21.99 ^b	1180.71 ±29.35 ^b	1435.71 ±21.56 ^b	1671.43 ±21.04 ^b
2	35.42 ±1.14 ^a	96.67 ±3.76 ^b	251.11 ±12.14 ^b	458.13 ±10.77 ^b	684.29 ±11.39 ^b	962.14 ±20.32 ^b	1210.71 ±28.31 ^b	1512.86 ±25.53 ^b	1762.86 ±22.55 ^b
3	35.00 ±0.87 ^a	94.58 ±5.05 ^b	250.56 ±8.91 ^b	451.25 ±11.87 ^b	691.00 ±13.60 ^b	966.43 ±22.41 ^b	1229.29 ±21.37 ^b	1525.00 ±25.92 ^b	1810.00 ±20.40 ^b
4	34.58 ±0.96 ^a	94.58 ±5.42 ^b	218.89 ±10.89 ^c	401.88 ±14.13 ^c	618.57 ±13.87 ^d	846.43 ±23.68 ^c	1028.57 ±23.17 ^c	-	-
5	35.42 ±0.96 ^a	114.58 ±3.91 ^a	283.89 ±7.35 ^a	502.50 ±7.50 ^a	765.00 ±14.60 ^a	1019.29 ±22.94 ^a	1425.00 ±22.27 ^a	1835.00 ±25.33 ^a	2150.00 ±24.55 ^a

Means within the same column carrying different superscripts are sig. different at $P < 0.05$ based on Tukey's Honestly Significant Difference (Tukey's HSD) test. W: week

Antibody (Ab) response

As shown in Table 2, under the impact of CIAV infection, the vaccination with live NDV vaccines (group I) exerted a very low HI Ab response in comparison with experimental chicks of group II and III which were vaccinated with combination of live and inactivated ND vaccines. This reduction was significant ($P < 0.05$) at 14, 28 and 35 days of age compared with group II as well as at 14,

21, 28 and 35 days of age compared with group III. Experimental chicks of groups II and III exhibited higher and significant increase ($P < 0.05$) in the Geometric Mean Titers (GMTs) at 28 and 35 days of age compared with the other groups. Moreover, birds of group III exhibited significant increase ($P < 0.05$) in the GMTs at 21 days of age in comparison with groups I and IV.

Table (2): The HI Ab response of chickens experimentally infected at one-day old with CIAV strain and challenged at 39-day old with vvNDV

Group	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days
1	10.00 ±0.00 ^a	3.33 ±1.67 ^b	8.33 ±1.67 ^b	6.67 ±1.67 ^c	5.00 ±2.89 ^b	213.33 ±53.33 ^{ab}	266.67 ±53.33 ^a	133.33 ±26.67 ^a
2	11.67 ±4.41 ^a	10.00 ±0.00 ^a	13.33 ±3.33 ^{ab}	53.33 ±13.33 ^a	66.67 ±13.33 ^a	293.33 ±174.87 ^a	320.00 ±160.0 ^a	213.33 ±53.33 ^a
3	13.33 ±3.33 ^a	10.00 ±0.00 ^a	20.00 ±0.00 ^a	26.67 ±6.67 ^b	66.67 ±13.33 ^a	266.67 ±53.33 ^a	373.33 ±106.6 ^a	213.33 ±53.33 ^a
4	8.33 ±1.67 ^a	6.67 ±1.67 ^{ab}	5.33 ±4.01 ^b	3.33 ±1.67 ^c	1.67 ±1.67 ^b	46.67 ±17.64 ^{ab}	-	-
5	11.67 ±4.41 ^a	8.33 ±1.67 ^a	6.67 ±1.67 ^b	5.00 ±0.00 ^c	3.33 ±1.67 ^b	1.67 ±1.67 ^b	-	-
6	11.67 ±4.41 ^a	8.33 ±1.67 ^a	6.67 ±1.67 ^b	5.00 ±0.00 ^c	3.33 ±1.67 ^b	53.33 ±13.33 ^{ab}	-	-

Means within the same column carrying different superscripts are sig. different at $P < 0.05$ based on Tukey's Honestly Significant Difference (Tukey's HSD) test.

Phagocytic activity

As indicated in Table 3, both the phagocytic percent and index were severely deteriorated post CIAV infection. The phagocytic percent of group I was remarkably and significantly decreased ($P < 0.05$) post CIAV infection at 2nd, 3rd and 4th week PI in comparison with experimental chicks of group V. Meanwhile, the phagocytic percent of experimental chicks of groups II and III were remarkably significantly decreased ($P < 0.05$) at 1st, 2nd, 3rd and 4th weeks PI in comparison with the non CIAV infected non ND vaccinated group (V). In addition, in case of post CIAV infection and NDV vaccination, the phagocytic percent of groups I, II and III had a

significant increase ($P < 0.05$) from the 8th and up to the 29th days of age in comparison with the CIAV infected and NDV non vaccinated group (IV).

The phagocytic index of experimental chicks of groups I, II and III were remarkably and significantly decreased ($P < 0.05$) post CIAV infection from the 8th up to the 29th days of age in comparison with the non CIAV infected group (V). The phagocytic index of experimental chicks of groups I, II and III had a significant increase ($P < 0.05$) from the 8th up to the 29th days of age in comparison with the CIAV infected and NDV non vaccinated group (IV).

Table (3): Effect of CIAV infection on the phagocytic activity of different NDV vaccination programs

Group	1 st w		2 nd w		3 rd w		4 th w	
	%	Index	%	Index	%	Index	%	Index
1	61.00 ±0.58 ^{ab}	3.20 ±0.06 ^c	59.33 ±0.88 ^c	3.13 ±0.03 ^c	61.67 ±0.88 ^c	3.23 ±0.09 ^c	59.67 ±0.88 ^c	3.07 ±0.09 ^c
2	60.00 ±0.58 ^b	3.13 ±0.09 ^c	64.00 ±1.15 ^b	3.87 ±0.09 ^b	66.33 ±1.20 ^b	3.73 ±0.09 ^b	65.00 ±1.15 ^b	3.33 ±0.09 ^b
3	60.33 ±0.88 ^b	3.50 ±0.06 ^b	63.33 ±0.33 ^b	3.70 ±0.06 ^b	65.67 ±0.88 ^b	3.67 ±0.12 ^b	65.00 ±0.58 ^b	3.50 ±0.15 ^b
4	43.33 ±1.45 ^c	1.57 ±0.09 ^d	41.33 ±0.88 ^d	1.33 ±0.09 ^d	47.00 ±0.58 ^d	1.60 ±0.06 ^d	50.00 ±0.58 ^d	1.80 ±0.06 ^d
5	63.67 ±0.88 ^a	4.60 ±0.06 ^a	67.67 ±0.88 ^a	4.87 ±0.12 ^a	70.33 ±0.88 ^a	4.93 ±0.09 ^a	70.67 ±0.33 ^a	4.97 ±0.12 ^a

Means within the same column carrying different superscripts are sig. different at $P < 0.05$ based on Tukey's Honestly Significant Difference (Tukey's HSD) test. W: week

Histopathological findings

The thymus of 7 days old chicks experimentally infected with CIAV showed medullary lymphoid depletion together with proliferation of the reticuloendothelial cells (Figure 2a). There was hyperplasia of lymphocytes in the cortex along with congestion of blood vessels in the medulla in some birds. Meanwhile, there was hyperplastic cortex and medulla in all lobules at 28 days old CIAV infected chicks represented by overpopulation of cells (Figure 2b). Severe lymphoid depletion of the splenic white pulps was detected in 7 days old chicks experimentally infected with CIAV (Figure

2c). There was moderate and severe hyperplasia of the white pulps along with proliferation of the reticuloendothelial cells throughout the splenic tissue at 28 days old (Figure 2d). Mild lymphocytic necrosis represented by karyorrhetic nuclei with homogenous cytoplasm was also seen. The spleen of some birds revealed thickening in the wall of some splenic arterioles. Bursa of Fabricius of 7 days old chicks experimentally infected with CIAV showed medullary and cortical depletion of lymphocytes and interfollicular fibroplasia (Figure 2e). A few lymphoid follicles of 21 days old chicks experimentally infected with CIAV showed necrosis of the lymphocytes represented by

karyorrhetic nuclei with homogenous and eosinophilic cytoplasm and their covering epithelium showed vesicle formation (Figure 2f). Inter and intrafollicular heterophilic infiltration and congested blood vessels were also detected. The bone marrows of 7 days old chicks experimentally infected with CIAV showed severe hypoplasia of the

hematopoietic tissue (Figure 2g). A number of birds showed moderate hypoplasia with proliferation of adipocytes replacing the normal tissue. There was regeneration of the hematopoietic tissue of the bone marrow in 28 days old chicks experimentally infected with CIAV (Figure 2h).

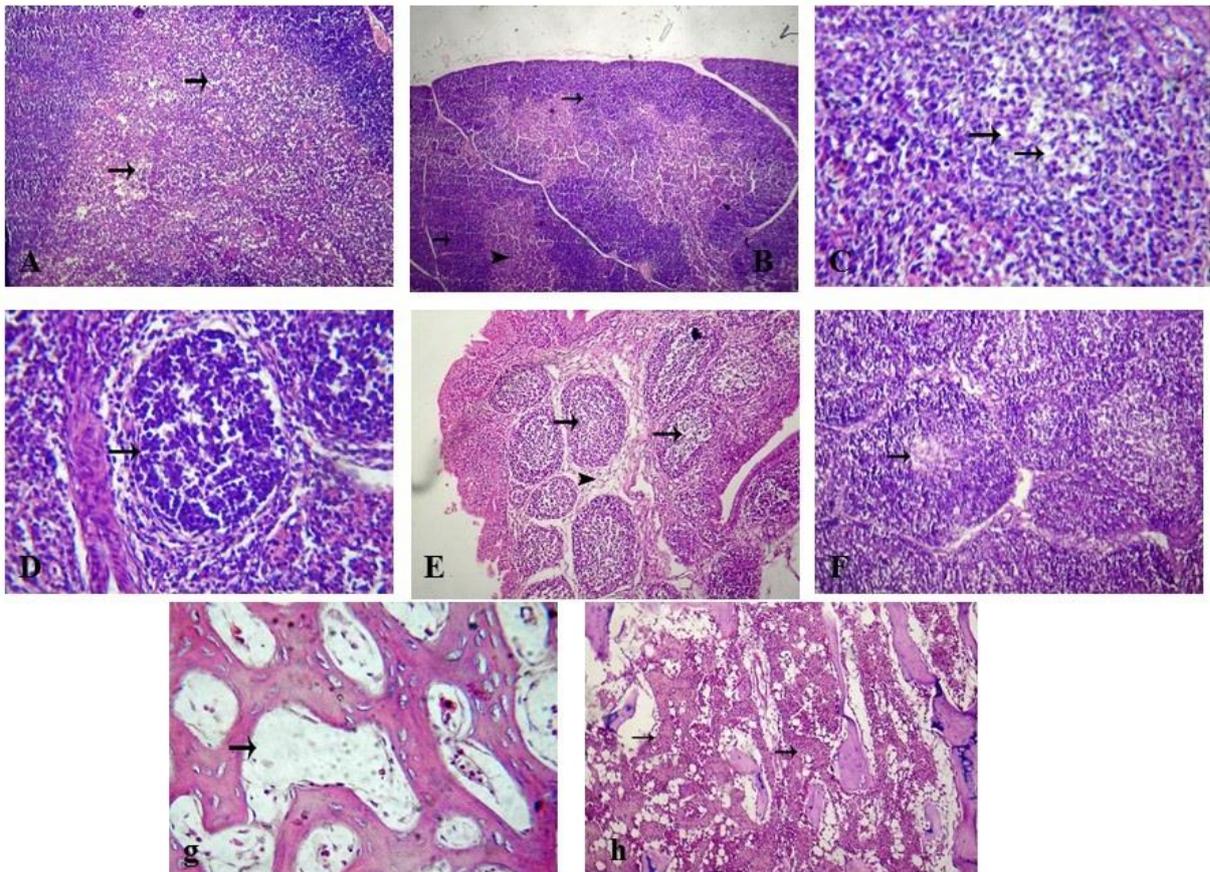


Figure (2): Thymus of 7 days old chicks experimentally infected with CIAV showing marked lymphocytic depletion from the medulla (arrow), H & E x300 (a). Thymus of 28 days old chicks experimentally infected with CIAV showing hyperplasia in the cortex (arrows) and medulla (head arrow), H & E x300 (b). Spleen of 7 days old chicks experimentally infected with CIAV showing severe depletion of lymphocytes (arrows), H & E x1200 (c). Spleen of 28 days old chicks experimentally infected with CIAV showing severe hyperplasia of the white pulps of spleen (arrow), H & E x1200 (d). Bursa of Fabricius of 7 days old chicks experimentally infected with CIAV showing severe depletion of lymphocytes from the bursal follicles (arrows) besides interfollicular fibroplasia (head arrow), H & E x300 (e). Bursa of Fabricius of 21 day showing mild focal necrosis of lymphocytes in the center of the follicles of bursa of Fabricius, H & E x1200 (f). Bone marrow of 7 days old chicks experimentally infected with CIAV showing marked decrease in the number of hematopoietic cells (arrow), H & E x1200 (g). Bone marrow of 28 days old chicks experimentally infected with CIAV showing normal appearance and architecture (arrows), H & E x300 (h).

Discussion

Virus induced immunosuppression is a recurring economic problem in the commercial poultry flocks [19]. Chicken infectious anemia virus is among the naturally occurring viruses that cause immunosuppression in chickens [3]. This virus may induce suppression in the differentiation and proliferation of haematopoietic precursor cells, thereby leading to transient destruction of erythroblastoid and granuloblastoid cell lineages in the bone marrow [13]. Therefore, it has a consequent negative impact on the bird immune system and leads to vaccination with sub-optimal antibody responses [8,9,20].

In the present study, the impact of CIAV infection on both NDV vaccination immune responses and bird performance was demonstrated. The infection with CIAV led to development of the clinical signs of the virus which was characterized by dullness, depression, diarrhea, ruffled feather and low feed intake in the infected birds fourteen days PI [21,22]. The observed dullness and depression in the infected birds could be attributed to the previously reported anemia development with the decrease of hematocrit value [9].

The gross pathology of the sacrificed birds, one-week PI, revealed specific postmortem lesions of the CIAV which was characterized by paleness of the breast muscles, icteric liver, atrophy of the thymus loops, pink color bone marrow and hemorrhage on the breast muscles [12,23]. The presence of hemorrhage in case of CIAV infected birds can be explained by the presence of thrombocytopenia, endothelial lesion and liver function impairment of the CIAV infected birds [24].

Interestingly, in the present study the mean live body weights and weight gains of the CIAV-infected and ND-vaccinated groups showed improvement compared with the CIAV infected but non-NDV vaccinated group. Therefore, it can be illuminated that the presence of NDV vaccine can contribute in the improvement of bird performance [21,25]. The entire CIAV-infected groups showed reduction

in the live mean body weight and weight gain compared with the non-infected group. This negative impact on the bird performance parameters could be explained by the observed low feed intake of the infected birds which may be attributed to the CIAV infection.

In the current study, the NDV specific HI antibody titers of the CIAV infected birds were assessed against different NDV vaccination programs. Different NDV vaccination programs were applied in order to imitate the field conditions and set a small experimental model. The NDV vaccines were previously used in several immunological studies as a reliable and easy marker for estimating the humoral immune responses [2,21,25]. The CIAV infected and non-NDV vaccinated group (IV) showed a marked reduction and nearly absence of the NDV-HI antibody titers compared with the other infected and vaccinated groups (I, II and III). The impact of CIAV infection was higher on live ND vaccinated group (I) as this group showed very minor HI-NDV Ab response in all the measuring points compared with the other vaccinated groups (II and III). The vaccination with combination of live and inactivated vaccines showed better HI-NDV Ab responses especially when inactivated vaccine was administrated at 9 days (group II). Hence, this group had statistically significant and vvNDV-protective HI-Ab titers at 28 days, compared with group III. These results were compatible with other previously reported studies [21,26]. A combination of the live and inactivated ND vaccines was recommended before use for adequate and protective immunization [3,13]. The dual vaccination with both live and inactivated NDV vaccines at different time points showed superior protection rate (100%) compared with the other groups [27]. On the other hand, vaccination with live vaccine only displayed moderate protection rate (75%) [21]. However, the non-vaccinated groups displayed inferior protection rate. It has been previously reported that vaccination program included inactivated NDV vaccine provided the birds with stringent and more effective immune response than

vaccination program that included live NDV only [28].

The results of the phagocytic activity test showed that the CIAV infected group was the lowest and statistically significant group in comparison with the non-infected group, which highlights the role of CIAV infection in suppressing the phagocytic activity of the bird [29]. Moreover, the results of histopathological examination of the experimentally infected chickens showed mild, moderate to severe changes in the lymphoid tissues (thymus, bursa, spleen and bone marrow). These changes were in the form of focal to diffuse depletion of lymphocytes and hematopoietic cells. Marked degeneration and necrosis of lymphocytes within the lymphoid tissues could be attributed to the specific tropism of CIAV for lymphoid tissues, particularly the thymus cortex which in turn affects lymphopoiesis causing depletion of thymic lymphoblastoid cells and lymphocytes in the thymus, spleen and other lymphoid tissues [30-32].

Conclusion

It could be concluded that CIAV infection has a negative impact on immunization against NDV as well as body performance parameters. This negative impact on NDV immunization was evident on both humoral and cell mediated immune responses. Chickens vaccinated with combination of live and inactivated NDV vaccines induced higher and long lasting immunity than vaccination with live vaccine only. Further studies are required to investigate the situation of CIAV infection in the Egyptian poultry farms.

Conflict of interest

None of the authors have any conflict of interest to declare.

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الملخص العربي

الأثر السلبي لعدوى فيروس انيميا الدجاج المعدى على الاستجابات المناعية للبرامج المختلفة لتحسين مرض فيروس النيوكاسل

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يعتبر فيروس انيميا الدجاج المعدى هو من بين الفيروسات التي تحدث بشكل طبيعي والتي قد تسبب نقص المناعة في الدجاج. فقد اظهرت هذه الدراسة اثر فيروس انيميا الدجاج المعدى على كلا من الاستجابات المناعية للتحسين ضد مرض النيوكاسل معدلات النمو. فقد اثبتت الدراسة انه بعد العدوى بفيروس انيميا الدجاج المعدى قد وجد تحسنا ملحوظا في متوسط الاوزان والاوزان المكتسبة للمجموعات المحصنة ضد مرض النيوكاسل عن غيرها الغير محصنة في باقى المجموعات. كما اظهرت الدراسة ان تأثير الفيروس المثبط للمناعة كان اكثر وضوحا في المجموعة الاولى المحصنة بالتحسين الحي فقط ضد مرض النيوكاسل مقارنة بباقى المجموعات المحصنة بكل من التحسين الحي والميت. كما اتضح من خلال الدراسة ان التحسين بكل من التحسين الحي والميت ضد مرض النيوكاسل اسفر عن اعلى استجابات مناعية خاصة عند استخدام التحسين الميت في اليوم التاسع من العمر. كما في المجموعة الثانية. كما اظهرت النتائج ايضا ان استخدام كلا من التحسين الحي والميت ضد مرض النيوكاسل اعطى اعلى نسبة حماية في اختبار التحدى تصل الى ١٠٠٪ مقارنة بباقى المجموعات الاخرى. مجهريا اظهرت النتائج ان العدوى بفيروس الدجاج المعدى ادت الى تدهور شديد في الأنسجة الليمفاوية على هيئة استنزاف يؤرى الى منتشر للخلايا الليمفاوية والخلايا المكونة للدم. واخيرا فقد القت هذه الدراسة الضوء على الآثار السلبية لعدوى فيروس انيميا الدجاج المعدى على كل من اداء الطيور والاستجابات المناعية.