EFFECT OF ACIDIFIERS AS AN IMMUNOSTIMULANT ON CHICKENS VACCINATED WITH INFECTIOUS BURSAL DISEASE VIRUS

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ABSTRACT

The present work was planned to study the ability of Acidifiers to overcome the immuno-suppressive effect of infectious Bursal Disease virus (IBDV) live vaccine on chickens vaccinated with Newcastle disease virus (NDV) live vaccines. The obtained results revealed that administration of acidifiers (3 ml/ liter of drinking water for the first 5 days of age and repeated for 3 successive days at 21 days of age) improved development of immunity to IBDV. At the same time, the immunosuppressive effect on NDV vaccination due to IBDV vaccination had been overcome after acidifiers treatment. The humoral immune response determined by Serum Neutralization test (SNT) and Haemagglutination test (HI test) and the cell mediated immune response determined by Lymphocyte blastogenesis. Results of neutralizing antibody revealed that the recorded titer for group I that vaccinated with IBDV only ranged between 16 at the first week post vaccination to 32 at 3rd week post vaccination. In the same time the neutralizing titer of the third group that vaccinated simultaneously with both live NDV and IBDV was ranged between 2 to 8 at 3rd week post vaccination. While the fourth group that vaccinated simultaneously with both live NDV and IBDV and treated with acidifiers was ranged between 32 to 64 at 3rd week post vaccination. The effect of acidifiers on the average Log2 HI antibody titer against NDV revealed that the best response was obtained in the fourth group (treated with acidifiers) than other groups under the condition of this experiment.

INTRODUCTION

Exposure of birds to certain pathogens lead to reduction of their immune response to infection or vaccination with other pathogens, as Infectious Bursal disease virus (IBDV) (**Springer *et al.*, 1983**). IBDV causes severe damage to bursa and an immunosuppress ion, rendering the birds susceptible to other diseases or reducing its capability to response to vaccination with other viral vaccines (**Mazariegos *et al.*, 1990** and **Tsukamoto *et al.*, 1995**). So, the application of immunostimulant not only to

rise the resistance of birds but also to improve their immune response to vaccines was reported by (**Afify, 1990** and **Awaad *et al.*, 2000 a**). .

The routine use of drugs to modify or modulate an animals immuno-competence as part of therapeutic management of specific clinical conditions is still at a very preliminary stage in veterinary medicine (**Brander *et al.*, *1991***). **Awaad *et al.*, (*1999*)** proved the immunopotentiation of the weak organic acids preparation for chickens using both immuno and Bioassays as criteria. Also **Awaad *et al.*, (2000 b)** proved the immunomodulation of acidifiers in cyclophosphamide treated chickens. The present investigation was planned to study the possible effect of acidifiers on the immune response of chickens vaccinated with IBDV vaccine as well as the ability of acidifiers to overcome the immuno-suppressive effect of IBDV live vaccine on chickens vaccinated with NDV live vaccines.

MATERIAL AND METHODS

I- Materials:

1. Embryonated Chicken Eggs (ECE): 9-11 days old fertile chicken eggs were obtained from commercial farm and used for preparation of NDV (HA) antigen, Titration of used viruses as well as virus reisolation from dead challenged birds.

2. Experimental birds: A total of one hundred and fifty (150), 1 day-old Hubbard chicks were divided into five equal groups; (30 chicks each). All groups of chicks were reared under complete hygienic measures in isolated and disinfected floor pens. The used chicks were given adequate feed and water.

3. Viral strains:

(a) Vaccinal strains:

1- Infectious Bursal disease virus live vaccine: IBDV vaccine (Bursa vac. strain) locally prepared with a titer of $10^{7.5}$ EID_{50}/ ml was kindly supplied by the Vet. Serum and Vaccine Research Institute, Abbasia, Cairo.

2- Newcastle disease live vaccine: Locally prepared LaSota vaccine with a titer of $10^{9.9}$ EID_{50}/ ml. kindly obtained from Veterinary Serum Research Institute, Newcastle Disease Unite, Abbasia, Cairo.

(b) Virulent Strains: for challenge test

1- Infectious Bursal disease virus:

IBDV virulent strain IBDV (10^{8} ED_{50}/ ml) was kindly supplied by the Animal Health Research Institute Immunity Unite, Dokki, Cairo.

2- Newcastle disease virus:

A velogenic viscerotropic NDV having a titer of 10^{8} EID_{50} was obtained from Veterinary Serum Research Institute, Newcastle Disease Unite, Abbasia, Cairo.

4- Positive sera:

Positive sera against NDV and IBDV were obtained from Veterinary Serum Research Institute, Newcastle Disease Unite, Abbasia, Cairo. used as positive control.

5- Samples:

a. Clotted blood: Ten samples were randomly collected weekly from each group for serum collection. The collected serum samples were individually subjected for HI test to detect NDV antibodies while pooled serum samples were used for serum neutralization test used for IBDV.

b. Non-clotted blood: five blood samples were collected weekly on heparin for separation of lymphocytes.

6. Acidifiers Nutrilac: Nutrilac liquid produced by NUTRI- AD International, Belgium, Lot No. NLL9803 was used 3ml/ liter for the first 5 days of age repeated at 21 days of age for 3 successive days.

II- Methods:

1- Vaccination: Experimental chicks were received both LaSota and IBDV vaccines via drinking water, each bird received a vaccine dose containing EID_{50} of $10^{3..5}$ and 10^{3}/ml respectively.

2- Evaluation of immune response:

a- Humoral immune response:

Serum Neutralization Test: It was used for IBDV. This test was done according to the method described by **Anon, (1971)**.

Haemagglutination Inhibition (HI) test: The test was used for NDV, it was applied according to the standard procedure described by **Majiyagbe and Hitchner, (1977)**.

b- Cell mediated immune response:

Lymphocytic transformation test: This was applied after **Lucy, (1984)**. Separation of lymphocytes was adopted after **Boyum, (1968)**. Determination of viable cell number was carried out according to **Hanks and Wallace, (1958)**. Culturing of Lymphocytes was performed as described by **Confer *et al.*, (1981)** using phytohaemagglutinin-P at a concentration of 10 µg / well. Evaluation of lymphocyte blastogenesis response using modified MTT dye uptake assay was adopted after **Garn *et al.*, (1994)**. The response of lymphocyte was given in terms of stimulation index according to **Carpenter *et al.*, (1978)**.

Challenge test against IBDV: 15 birds from each group were subjected to challenge test against IBDV at 21 days post vaccination. Each bird was installed intraocularly with $10^{-4.7}$ dilution of virulent IBDV (10^{8} EID_{50}) according to (**Okoye and Uzoukwu, 1990**).

NDV challenge test:

15 birds from each group were challenged orally at 21 days post vaccination with virulent NDV (VVNDV) strain of a titer 10^6 EID_{50}/ ml.by a dose of 0.1ml according to (**Abou- Elkhair *et al.*, 1998**).

3- Bursal Lesion:

a- Bursal body weight ratio (B:B):

Bursa/body weight ratio were calculated as percentage for individual birds according to **Sharma *et al.*, (1989)** as follows:

$$\text{Bursa / body weight ratio} = \frac{\text{Bursa weight}}{\text{Body weight}} \times 100$$

b- Bursa body weight index: It was calculated according to **Sharma *et al.*, (1989)** as follows:

$$\text{Bursa body weight index} = \frac{\text{B/B of infected bird}}{\text{B/B of control bird}}$$

1- Experimental design:

The used 150, one-day old chicks were randomly divided into 5 equal groups, 30 chicks each. Each group 1- 5 were kept in separated, clean disinfected pens on floor. All chicken groups were given commercial ration without feed additives and clean water adlibitum. At 16th day of life when neutralization test and HI test proved undetectable amount of maternal antibodies against IBDV and NDV. Chicken group were treated as follows:

Group I: vaccinated with live IBDV vaccine only.

Group II: vaccinated with live NDV vaccine (LaSota strain) only.

Group III and IV: were simultaneously vaccinated with both live NDV and IBDV vaccines

Group V: were kept as control –ve (non-treated non-vaccinated).

- The used acidifiers was given to chicks of group 4 in a rate of 3 ml / liter of drinking water through the first 5 days of life and repeat for 3 successive days at 21 days of life.
- At weekly interval after vaccination 10 clotted blood samples were collected for serum separation and 5 non-clotted heparenized heart blood samples were collected for lymphocyte separation from each group.
- At 3rd week post vaccination each group (30 chicks) was randomly divided into 2 subgroups (15 bird each) and each subgroups were kept in isolated pen.
- One half of each mean group was challenged with IBD virulent virus while the other half were challenged with velogenic VNDV.

- The challenged birds were subjected for daily observation for clinical signs and mortailities as well as P.M. lesions for 7 and 15 days for IBDV and NDV challenged respectively.
- Both bursa and total body weight of dead and sacrificed birds at the end of observation were weighted to calculate both B:B ratio and index.

RESULTS

Results are shown in Table (1) neutralizing antibody revealed that the recorded titer for group I that vaccinated with IBDV only ranged between 16 at the first week to 32 at the 3rd week post vaccination. In the same time the neutralizing titer of the 3rd group that vaccinated simultaneously with both live NDV and IBDV was ranged between 2 at the first week to 8 at the 3rd week post vaccination. While the 4th group that treated with acidifiers showed the highest neutralization titer was ranged between 32 at the first week to 64 at the 3rd week post vaccination.

Results of HI antibody titer against NDV, as shown in Table (2) revealed that the best response was obtained in group 4 (treated with acidifiers) followed by group 2 and 3, where the recorded peak titers were 7.2, 6.0 and 4.3 that recorded at the 3rd post vaccination.

Results represented in Table (3) revealed that significantly higher stimulation index appeared in group (4) that treated with acidifiers at 3rd week post vaccination (2.5).

Results of challenge test against Newcastle as represented in Table (4) indicated that the highest protection percentage (93.3%) could be achieved on using acidifiers in group 4. Meanwhile, group 2 and group 3 gave 86.6% and 80.0% level of protection respectively, 0% level of protection was recorded for group 5 (non -vaccinated non -treated control group) and group I (vaccinated only with IBDV).

Challenged birds with NDV showed clinical symptoms at the 48 hours post challenge in group 5 including depression, and prostration marked respiratory signs that include gasping coughing nasal discharge. While mortalities started at the 72 hours post challenge in birds of group 5 with P. M. lesions mild airsaculitis, tracheitis, cecal tonsils are necrotic and hemorrhagic.

Results of challenge test against IBDV as represented in Table (5) indicated that full protection against challenge could be achieved on using acidifiers in group 4. Meanwhile, group 1, 3 gave level of protection 86.6% and 80.0% respectively. 0% level of protection was recorded for group 5 (non-vaccinated, non–treated control group and group II which vaccinated only with NDV.

Challenged birds with IBDV showed clinical symptoms at the 36 hours post challenge in group 5 including depression, anorexia, ruffled feathers and

droopy appearance. While mortalities started at the 48 hours post challenge in birds of group 5 with P. M. lesions of enlarged bursa, severely edematous and reddened contain hemorrhages kidneys swollen and ureters contain urates.

Results of bursa body weight indices in different groups of challenged birds as shown in Table (6) it was noticed that group 4 (treated with acidifiers) gave the highest mean value (1.32) followed by group 1 and group 3 where the obtained values were (1.02) and (0.86) respectively. Obtained values were considered within the normal level except in group 5 (non-vaccinated non- treated & challenged) mean value (0.46).

DISCUSSION

This study depended mainly upon using of acidifiers in trial to overcome the possible immunosuppressive effect of IBDV live vaccine on chicken vaccinated simultaneously with ND live (LaSota) vaccine. Results of neutralizing antibody as represented in Table (1) revealed that the recorded titer for group I that vaccinated with IBDV only ranged between 16 at the first week to 32 at the 3rd week post vaccination. In the same time the neutralizing titer of the 3rd group that vaccinated simultaneously with both live NDV and IBDV was ranged between 2 at the first week to 8 at the 3rd week post vaccination.

Dealing with the effect of acidifiers and IBDV vaccination on the average Log2 HI antibody titer against NDV. Table (2) revealed that the highest titer were obtained in group 4 followed by group 2 and 3, where the recorded peak titers were 7.2, 6.0 and 4.3 that recorded at the 3rd week post vaccination, respectively.

From Tables (1 and 2), it could be noticed that birds vaccinated simultaneously with IBDV and NDV living vaccines showing low immunological response compared with the other groups and it could be attributed to the using of two live virus vaccines. IBDV causes severe damage to bursa and an immunosuppression, rendering the birds susceptible to other diseases or reducing its capability to response to vaccination with other viral vaccines **Mazariegos *et al.*, (1990)** and **Tsukamoto *et al.*, (1995)**. Also the observation agreed with that of **Sasaki *et al., (1969)* and Koichi and Yoshikazu (1973)** where they concluded that an interference occurs when different live viruses vaccines used together. However, this observation was overcome in the fourth group when acidifiers was used as an immunostimulant. Where the recorded neutralizing antibody titer for group 4 was ranged between (32) at the first week, reached its peak (64) at the 3rd week post vaccination, as well as the haemagglutinating antibody of this group recorded the highest titer (7.2) compared with the other groups.

On the other hand, a noticeable difference was found in the stimulation indices of lymphocyte blastogenesis between group 4 and the other groups.

Table (3) The highest stimulation index in group 4 (that treated with acidifiers) was (2.5) at 3rd week post vaccination followed by group 1, 2 and 3 respectively where the maximum response were 2.0, 2.0 and 1.8 at 3rd week post vaccination, respectively. Similar observation was recorded by **Awaad *et al.*, (2000)** where they found that stimulation indices of lymphocytic transformation measured by MTT revealed statistical significant increase in chickens received acidifiers. The results of both humoral and cellular immunity comes in contact with that of challenge test against NDV represented in Table (4) indicated that the highest protection percentage (93.3%) could be achieved on using acidifiers (group 4).

Results of challenge test against IBDV. Table (5) pointed out a full protection in group 4, while, group 1, 3 gave protection percentage 86.6% and 80.0% respectively. 0% protection percentage was recorded for group 5 (non-vaccinated, non –treated control group and group 2 (vaccinated with NDV only). The result indicated the immunostimulating effect of acidifiers in birds of group 4.

Dealing with the results of bursa body weight indices in different groups of challenged birds as shown in Table (6). It was noticed that group 4 (treated with acidifiers) gave the highest mean value (1.32) followed by group 1 and group 3 where the obtained values were (1.02) and (0.86) respectively versus (0.46) in non-vaccinated non- treated control group. Obtained values were considered within the normal level these results agree with **Lucio and Hitchner, (1978)** who reported that the bursa from chickens with B:B indices higher than 0.70 were found to be histologically normal. This results agree with **Awaad *et al.*, (2000)** who recorded that statistical significant increase in B/B. wt index in acidifiers treated group.

Regarding our findings and taking in consideration results of **Awaad *et al.*, (1999)**, it could be concluded that acidifiers has a stimulatory effect on both cell mediated and humoral immunity. Moreover; it could be concluded that it is not only a potent immunostimmulator but also a counter- attacking modulator that accomplish immune-stimulation and compensate immunosuppression. **Bradner *et al.*, (1991)** reported that immunostimulants exert their effects when administered prior to antigenic challenge and are useful for protecting immunocompromised animals at risk from opportunistic infections or, alternatively, animals that have been exposed to virulent infectious agents. On the other hand, immunomodulators administered simultaneously with antigens may prove to be effective immunologic adjuvant for the potentiation of a specific immune response, particularly to vaccines.

In conclusion, Nutrilac gave better humoral and cellular immune response and overcome the immuno-suppressive effect of infectious Bursal Disease virus (IBDV) live vaccine on chickens vaccinated with Newcastle disease virus (NDV) live vaccines.

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Table (1): Mean IBDV neutralizing antibody titers in sera of chickens vaccinated with IBD and NDV vaccines with or without treatment with acidifiers.

Groups of chicks	*Treatment*	*Mean neutralizing titer*		
		Weeks post vaccination		
		1st	*2nd*	*3rd*
Group I	Vaccinated with IBDV only	16	16	32
Group II	Vaccinated with NDV only	0	0	0
Group III	Simultaneously vaccinated with IBD and NDV	2	8	8
Group IV	simultaneously vaccinated with IBD, NDV and treated with acidifiers	32	64	64
Group V	control –ve (non vaccinated non treated)	0	0	0

Table (2): Average HI antibody titers (Log2) in sera of chickens vaccinated against ND and IBD vaccines with or without treatment with acidifiers.

Groups of chicks	*Treatment*	Mean neutralizing titer		
		Weeks post vaccination		
		1st	2nd	3rd
Group I	Vaccinated with IBDV only	0	0	0
Group II	Vaccinated with NDV only	2.25	5.8	6
Group III	Simultaneously vaccinated with IBD and NDV	1.75	4	4.3
Group IV	Simultaneously vaccinated with IBD, NDV and treated with acidifiers	2.50	6.25	7.2
Group V	control –ve (non vaccinated non treated)	0	0	0

Table (3): Immunomodulatory effect of acidifiers on lymphocyte transformation on chickens immunized with both IBDV and ND vaccine.

Groups of chicks	Treatment	Stimulation index of lymphocyte transformation Measured by MTT		
		Weeks post vaccination		
		1 studies	2nd	3rd
Group I	vaccinated with IBDV only	1.6 ± 0.03	1.8 ± 0.05	2.0 ± 0.05
Group II	vaccinated with NDV only	1.6 ± 0.05	1.8 ± 0.02	2.0 ± 0.05
Group III	Simultaneously vaccinated with IBD and NDV	1.4 ± 0.01	1.5 ± 0.03	1.8 ± 0.05
Group IV	Simultaneously vaccinated with IBD, NDV and treated with acidifiers	1.8 ± 0.03	2.2 ± 0.05	2.5 ± 0.06
Group V	control –ve (non vaccinated non treated)	1.8 ± 0.05	2.0 ± 0.03	2.1 ± 0.02

Table (4): Results of NDV Challenge test 3 weeks post vaccination.

Groups	No. of birds	Mortality	Protection %
Group I	15	15/15	0.0
Group II	15	2/15	86.6
Group III	15	3/15	80.0
Group IV	15	1/15	93.3
Group V	15	15/15	0.0

Table (5): Results of IBDV challenge test 3 weeks post vaccination.

Groups	No. of birds	Mortality rate	Protection %
Group I	15	2/15	86.6
Group II	15	15/15	0.0
Group III	15	3/15	80.0
Group IV	15	0/15	100
Group V	15	15/15	0.0

Table (6): Effect of Nutrilac on bursal body weight index of chicken vaccinated with IBDV vaccine after challenge with virulent IBD at the 3rd week post vaccination.

Groups	Treatment	Total body weight TBW/gm	Bursal weight BW/gm	BW/TBW ratio	B:B index	Mean BW/TBWs
Group I	vaccinated with IBDV only	850	1.105	0.0013	1.19	1.02
		830	0.748	0.0009	0.82	
		900	0.810	0.0009	0.82	
		790	0.791	0.0010	0.9	
		782	0.942	0.0012	1.1	
Group III	Simultaneously vaccinated with IBDV and NDV	770	0.700	0.0009	0.82	0.86
		790	0.791	0.0010	0.92	
		710	0.780	0.0011	1.01	
		730	0.585	0.0008	0.73	
		860	0.600	0.0007	0.64	
Group IV	Simultaneously vaccinated with IBDV and NDV and treated with acidifiers	900	1.532	0.0017	1.55	1.32
		870	1.305	0.0015	1.37	
		783	1.180	0.0015	1.38	
		890	0.977	0.0011	1.009	
		920	1.290	0.0014	1.28	
Group V	control non vaccinated non treated challenged birds	750	0.450	0.00060	0.55	0.46
		820	0.443	0.00054	0.46	
		750	0.450	0.00060	0.55	
		720	0.284	0.00039	0.36	
		8.20	0.443	0.00054	0.46	
Blank control	non vaccinated non treated non challenged	730	0.818	0.00112		0.00109
		780	0.827	0.00106		
		820	0.820	0.0010		
		940	1.092	0.0012		
		715	0.765	0.00107		

الملخص العربي

تأثير مستخلص البكتريا الحمضية كمحفز مناعى عند تحصين الكتاكيت ضد مرض الجمبورو

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تم أجراء هذه التجربة لدراسة قابلية مستخلص البكتريا الحمضية فى التغلب على التأثير المثبط المناعى للقاح مرض الجمبورو الحى فى الكتاكيت المحصنة ضد مرض النيوكاسل الحى وقد لوحظ أن النيترولاك محفز مناعى ضد التحصين لمرض الجمبورو. وقد ثبت من هذه الدراسة أن إضافة النيترولاك إلى مياه الشرب للكتاكيت المحصنة بلقاحى الجمبورو والنيوكاسل أدى ألى ارتفاع معدل الكفاءة المناعية الخلوية والخلطية لكلا اللقاحين بالمقارنة بالمجاميع الأخرى الغير معاملة بالنيترولاك تحت ظروف هذه التجربة.