EFFECT OF POST VACCINATION MEDICATION ON LAYER CHICKS VACCINATED WITH GUMBORO VACCINE NOBILIS D-78

S.A. Khan, S.M. Subtain, A. Aslam, K. Muhammad[1] and K.A. Khan
Department of Pathology, [1]Department of Microbiology,
University of Veterinary and Animal Sciences, Lahore, Pakistan

ABSTRACT

One hundred and sixty one-day-old layer chicks were divided into four experimental groups A, B, C and D, with 40 birds in each group. Group A was kept as control (non-vaccinated), group B was given vaccine but not medicated, group C was administered vaccine as well as multivitamins for 3 days post-vaccination, while group D was also medicated with aspirin for 3 days post-vaccination. The parameters studied were: heterophil/lymphocyte ratio, serum biochemical analysis (serum protein, glucose and cholesterol), antibody response against infectious bursal disease virus (IBDV). At the end of experiment (42nd day) adrenal glands from 10 randomly selected birds from each group were subjected to gross and histopathological examination and adrenal/body weight ratio was also determined. The results showed non significant difference among different groups. However, the group that was given multivitamins showed maximum immune response against IBDV, while the aspirin therapy did not show any significant difference. It can be concluded that vaccine produced undetectable stress in layer chicks and the vitamin supplementation evidently showed as an immuno-potentiating effect.

Key words: Stress, vaccination, infectious bursal disease, multivitamins, acetyl salicylic acid

INTRODUCTION

The layer farming is approaching towards progress in Pakistan despite some major problems such as increasing production costs and decreasing profit margins, especially due to a high mortality rate (Qureshi, 1999). Among the diseases that cause major economic losses, viral infections are most important and for their control therapeutic agents are of no value and hygiene precautions with the aim of eradicating infection on premises are often unsuccessful, even with depopulation, cleansing and disinfection. In practice, control of viral diseases depends upon the production of active or passive immunity by vaccinating either chicks with live virus vaccines or hens with live, inactivated or both types of vaccines.

Alongwith protection, which is a beneficial effect, vaccination can cause stress in poultry birds (Duneva and Dimitrova, 1987; Saxena, 1997). Stress is a systemic state that develops as a result of short and long term application of stressors. From an ethological standpoint, stress is manifested by abnormal behaviour patterns such as increase in aggression or frustration (Duncan and Wood-Gush, 1971). Vaccination, with live intermediate vaccine, against infectious bursal disease can also cause immunosuppression and bursal atrophy (Mazariegos *et al*., 1990) that may lead to stress condition.

Keeping in view the frequent use of vaccines in layer farming, this study was designed to develop measures for management in layer chicks following vaccination against infectious bursal disease (IBD). The results of this study will hopefully help to suggest post-vaccination medication, which will in turn improve the health status of the flock.

MATERIALS AND METHODS

Experimental birds

One hundred and seventy one-day-old layer chicks were obtained from local market. Ten chicks were used to collect blood samples to detect maternal antibody level against infectious bursal disease virus (IBDV) by cardiac puncture on the first day of the experiment. The remaining 160 layer chicks were reared upto 42nd day of age, under standard experimental conditions during March and April, 2000. The birds were fed commercial feed and water *ad-libitum* and were vaccinated against Newcastle disease.

Experimental materials

a) Gumboro vaccine Nobilis strain D-78 (Vety. Care) live, freeze dried vaccine against IBD.
Route: Orally in drinking water on 16th day of age.
b) Vety-stresscheck (Vetycare) containing multi-vitamins.

Dose: 1 gm/ 4 lit. of drinking water.

c) Asprin (Shamsi Pharma) containing acetyl salicylic acid.
Dose: 180 mg/ lit. of drinking water.

Experimental design

Chicks were randomly divided into four groups (A, B, C and D) having 40 chicks each on day-4 of the experiment. Experimental design is shown in Table 1.

Collection of samples

Blood samples from 10 randomly selected birds of each group were collected on day 1, 2 and 3 post-vaccination and serum was separated for estimation of serum biochemical compounds. Fresh blood smears from all samples were also prepared for determination of heterophil/lymphocyte ratio. Blood samples were also collected from 10 birds of each group on day 14, 28 and 42 of experiment for determination of antibody response against IBDV. Adrenal glands were collected after slaughtering at 42nd day of the experiment.

Experimental parameters

The following parameters were studied for chicks of each group.

a) Heterophil/lymphocyte ratio (Benjamin, 1978).
b) Serum biochemical substances i.e. total serum protein (Gornall *et al*., 1949), serum glucose (Somogyi, 1946) and serum cholesterol (Coles, 1986).
c) Adrenal/body weight ratio.
d) Gross and histopathological examination of adrenal gland (Drury and Wallington, 1980)
e) Antibody response against IBDV by IHA test (Liu and Gao, 1989).

Statistical analysis

Data thus collected were analyzed statistically by applying one-way analysis of variance (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Results of this study are tabulated in Tables 2-7. It was found that the heterophil/lymphocyte ratio of the vaccinated birds of group B, which was higher than that of other groups, showed no significant difference ($P>0.05$) among the birds of groups A, B, C and D on the first, second and third day post-vaccination (Table 2). Some workers had observed significant difference ($P<0.05$) in heterophil/ lymphocyte ratio (Jones, 1989; McFarlane and Curtis, 1989). It has been reported that hypothalmus is activated due to effect of stressor. It activates the pituitary gland to increase production of the adrenocorticotropic hormone (ACTH) which affects lymphoid tissues and causes decrease in lymphocytes and increase in heterophilic granulocytes. Severe stress produces high level of ACTH that causes significant difference in heterophil/lymphocyte ratio while mild type of stress causes transient change. Our findings do not agree with them, but are similar to those of Freeman and Manning (1984), who reported about failure to induce stress reactions following vaccination against Marek's disease (MD) or Newcastle disease (ND).

The mean total serum protein levels of vaccinated groups B, C and D were lower from the non-vaccinated group A, on day first to third post-vaccination. But when statistically analyzed, there was no significant difference ($P>0.05$) among four groups (Table 3). These results correlate with Freeman and Manning (1984) and Guyton and Hall (1996). But our results differ from those of Al-Afaleq (1998) and Puvadolpirod and Thaxton (2000), who reported decrease in total protein due to administration of ACTH. A catabolic effect (negative nitrogen balance) occurs after treatment with adrenocortical hormones or with sufficient level of ACTH. It also reduces synthesis of proteins, especially of structural and muscle proteins. This all occurs with high level of ACTH in blood, but low or normal level of ACTH does not produce significant alteration in total serum protein level, as observed in our study. The other reason could be the use of multi-vitamins in one vaccinated group. Duneva and Dimitrova (1987) recorded that addition of crude protein and vitamin A minimized the stress by increasing the serum protein in the blood of chicken, but we used multi-vitamins with different dose. This could be the possible reason that group with multi-vitamin showed no significant difference with other groups.

The mean serum glucose level of control (non-vaccinated) group showed no significant difference ($P>0.05$) with vaccinated groups on day 1, 2 and 3 post-vaccination (Table 4). Freeman *et al*. (1979) reported that serum glucose level was within the normal range 24 hours after the injection of corticotropin. Freeman and Manning (1984) and Khaliel and El-Manakhly (1998) noted a transient change in glucose level following vaccination against ND.

The mean serum cholesterol level of group B was elevated as compared to that of groups A, C and D. However, the statistical analysis showed non-significant difference ($P>0.05$) between the groups on first, second and third day post-vaccination (Table 5). Similarly, Freeman and Manning (1984) reported non-significant difference in plasma cholesterol after vaccination against Marek's disease.

Table 1: Experimental design

Treatment	Experimental day	Groups			
		A	B	C	D
Vaccination against IBD	16th	–	+	+	+
Vety–stresscheck	16-19th	–	–	+	–
Aspirin	16-19th	–	–	–	+

Table 2: Mean values (± SE) of heterophil /lymphocyte ratio of experimental and control groups

Groups	Post-vaccination period (Day)		
	1st	2nd	3rd
A	0.43 ± 0.01	0.43 ± 0.01	0.46 ± 0.01
B	0.48 ± 0.01	0.48 ± 0.04	0.49 ± 0.01
C	0.44 ± 0.04	0.45 ± 0.01	0.44 ± 0.02
D	0.46 ± 0.01	0.47 ± 0.02	0.48 ± 0.01

Mean values on day 1, 2 and 3 post vaccination do not differ statistically among four groups.

Table 3: Mean values (± S.E.) of total serum protein level of experimental and control groups (mg/dl)

Groups	Post-vaccination period (Day)		
	1st	2nd	3rd
A	7.92 ± 0.09	7.58 ± 0.24	7.60 ± 0.19
B	6.84 ± 0.09	7.05 ± 0.16	7.02 ± 0.20
C	6.97 ± 0.07	6.75 ± 0.19	6.75 ± 0.14
D	7.00 ± 0.24	6.81 ± 0.27	6.25 ± 0.15

Mean values on day 1, 2 and 3 post vaccination do not differ statistically among four groups.

Table 4: Mean values (± SE) of total serum glucose level of experimental and control groups (mg/dl)

Groups	Post-vaccination period (Day)		
	1st	2nd	3rd
A	178.55 ± 1.86	187.80 ± 1.54	181.96 ± 2.40
B	183.46 ± 1.45	192.78 ± 1.30	188.23 ± 3.10
C	179.95 ± 1.92	185.15 ± 1.44	183.15 ± 2.31
D	180.01 ± 1.86	190.15 ± 2.11	178.24 ± 2.67

Mean values on day 1, 2 and 3 post vaccination do not differ statistically among four groups.

Table 5: Mean values (± SE) of total serum cholesterol of experimental and control groups (mg/dl)

Groups	Post-vaccination period (Day)		
	1st	2nd	3rd
A	132.29 ± 3.19	136.09 ± 3.03	134.09 ± 2.79
B	140.15 ± 2.96	142.19 ± 3.04	138.52 ± 3.34
C	135.35 ± 3.84	140.02 ± 3.06	130.13 ± 2.56
D	136.50 ± 3.39	130.30 ± 4.62	132.57 ± 3.59

Mean values on day 1, 2 and 3 post vaccination do not differ statistically among four groups.

Table 6: Mean values (± SE) of adrenal /body weight ratio of experimental and control groups

Groups	Adrenal/body weight ratio
A	0.0098 ± 0.0076
B	0.0109 ± 0.0016
C	0.0103 ± 0.0024
D	0.0102 ± 0.0028

Mean values among four groups do not differ statistically.

Table 7: Antibody titers of birds of control and experimental groups to IBDV

Groups	IHA Geomatric Mean Titer (GMT) on day			
	1	14	28	42
A	22.6	5.7	2.5	1.6
B	22.6	5.7	44.2	62.4
C	22.6	9.0	58.4	70.5
D	22.6	5.4	46.0	58.6

The mean adrenal gland/body weight ratio of the experimental groups A, B, C and D showed non-significant difference ($P>0.05$) when analyzed statistically (Table-6). This supports the findings of Freeman and Manning (1982, 1984), who stated that adrenal weight determination as a stress indicator was unreliable. The results of our study differ from Conner (1959), who reported enlargement of adrenal due to severe stress. But in our study vaccination stress was mild in nature, which probably produced no significant increase in the level of ACTH, thus not causing hypertrophy of gland.

On gross and histopathological examination of adrenal glands of different experimental groups, no specific lesions were observed. But non-specific lesions such as slight variations in colour, size and appearance were observed. The main factor, which causes change in the tissue of gland is release of ACTH that produces an increase in cyclic adenomonophosphate (AMP) by stimulating adenyl cyclase thus producing molecular changes in the adrenal glands. No such changes were detected in our study. The reason might be use of mild type of stressor (vaccination stress with live virus).

The idea behind vaccination is to develop immunity by giving a stimulus of mild form of antigen, which produces antibodies. Best vaccine reflects best antibody titre. Our results explain that GMT was the highest in the experimental group C on all experimental days (Table 7). This may be due to supplementation of multi-vitamins to group C. Ferket and Qureshi (1992) and Shadaksharappa *et al*. (1998) reported that vitamins therapy had a positive effect on the development of antibody titre. Our results are in line with the above workers. From our study it can also be speculated that aspirin therapy in vaccinated group D did not show any advantageous effect on the development of immunity against IBDV.

On the basis of this study, the following points can be concluded and suggested:

1. Gumboro vaccine Nobilis strain D-78 did not cause considerable stress in birds but conferred a significant antibody titre against IBDV.
2. The role of multi-vitamins is encouraging in development of immunity.

In future, the experiment can be repeated with the following modifications:

1. The detection of stress be conducted by using more than one vaccine.
2. Detection of stress hormones be indicated.
3. Morphological changes in adrenal gland be examined immediately after the application of stressor.

REFERENCES

Al-Afaleq, A.I., 1998. Biochemical and hormonal changes associated with experimental injection of chicks with infectious bursal disease virus. J. Vet. Med., Series-B, 45(9): 513-517.

Benjamin, M.M., 1978. Outline of Veterinary Clinical Pathology, 3rd Ed., Iowa State University Press, U.S.A., pp. 43-47.

Coles, E.H., 1986. Carbohydrate metabolism and pancreatic function. Vet. Clinical pathology, 4th Ed. W.B. Saunder Company, Philodelphia, USA pp. 139-193.

Conner, M.H., 1959. Effect of various hormone preparations and nutritional stresses in chicks. Poult. Sci., 38: 1340-1343.

Drury, R.A.B. and E.A. Wallington, 1980. Carleton's Histopathological Techniques, 5th. Ed; Oxford Univ. Press, UK. pp. 39-115.

Duncan, I.H. and D.G.M. Wood-Gush, 1971. Frustration and aggression in domestic fowl. Anim. Behav., 19: 500-504.

Duneva, H. and E. Dimitrova, 1987. Effect of the amount of crude protein and vitamin A in mixed feeds for breeding broiler chickens on immunity stress to Newcastle disease. Zhivotnov dni-Nauki, 24(12): 86-93.

Ferket, P.R. and M.A. Qureshi, 1992. Performance and immunity of heat stressed broilers fed vitamin and electrolyte-supplemented drinking water. Poult. Sci., 71(1): 88-97.

Freeman, B.M., A.C.C. Manning and L.H. Flack, 1979. Habituation by the immature fowl in response to repeated injections of corticotrophin. British Poult. Sci., 20: 391-399.

Freeman, B.M. and A.C.C. Manning, 1982. An evaluation of adrenal mass and adrenal cholesterol as measures of adrenal activity. British Poult. Sci., 23: 257-262.

Freeman, B.M. and A.C.C. Manning, 1984. Failure to induce stress reactions following vaccination against Marek's disease or Newcastle disease. Res. Vet. Sci., 36(2): 247-250.

Gornall, A.G., E.J. Bardawill and M.M. David, 1949. Determinations of total serum protein by means of Biuret reaction. J. Biol. Chem., 177: 364-365.

Guyton, A.C. and J.E. Hall, 1996. Genetic control of cell function-protein synthesis and cell reproduction. Text Book of Medical Physiology, 9th Ed., W.B. Sounders Philadelphia, USA, pp. 916.

Jones, R.B., 1989. Chronic stressors, tonic immobility and leucocytic responses in the domestic fowl. Physiol. Behav., 47(3): 439-442.

Khaliel, S.A. and E.M. El-Manakhly, 1998. Pathologic, immunocytochemical and immunologic studies on a new infectious bursal disease vaccine "Intermediate plus" in chickens. Egyptian J. Comp. Pathol. Clin. Pathol., 11(2): 185-198.

Liu, Y. and X. Gao, 1989. Study on the detection of IBD antibody using indirect haemagglutination assay. J. Shanghai Agri. College, 7(1): 69-74.

Mazariegos, L.A., P.D. Lukert and J. Brown, 1990. Pathogenicity and immunosuppressive properties of infection assessing the economic effects of disease in broiler chicken flocks. Prev. Vet. Med. Ed., 2(3): 151-158.

McFarlane, J.M. and S.E. Curtis, 1989. Multiple concurrent stressors in chicks, 3. Effects on plasma corticosterone and the heterophil-lymphocyte ratio. Poult. Sci., 68(4): 522-527.

Puvadolpirod, S. and J.P. Thaxton, 2000. Model of physiological stress in chickens. 4. Digestion and metabolism. Poult. Sci., 39(3): 383-390.

Qureshi, A.A., 1999. Gumboro disease in Pakistan. Poultry International, 38(4): 42-43.

Saxena, H.C., 1997. Stress factors, major setback to the broiler industry. World Poult., 13(2): 36-37.

Shadaksharappa, H.L., R.N.S. Gowda and S.K. Vijayasarathi, 1998. Immunomodulatory effects of levamisole hydrochloride, vitamin E and vitamin C on immune response against infectious bursal disease (IBD) vaccination in broilers. Indian Vet. J., 75(5): 399-401.

Somogyi, J., 1946. Carbohydrate metabolism and function of pancreas and digestive tract. J. Biol. Chem., 16: 160-162.

Steel, R.G.D. and J.H. Torrie, 1982. Principles and Procedures of Statistics. A biochemical approach, 2nd. Ed. McGraw Hill Book Co. Inc., New York, pp. 137-140.