A STUDY ON THE IMMUNOMODULATING EFFECT OF GENTAMICIN ON BROILERS VACCINATED WITH NEWCASTLE DISEASE VIRUS

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ABSTRACT

The aim of the present work is to study immunomodulating effect of gentamicin on broiler chickens vaccinated against Newcastle virus at 18 days and challenged with a virulent strain of ND at the 30th day of age. Three groups of one day old Hubbard chicks each of 30 birds were used in this study. Chicks of the 1st group received 10 mg gentamicin /kg b. wt and those of the 2nd group were given 20mg gentamicin /kg b. wt . All the birds of the two groups were injected S/C at 14th,15th and 16th days of age .Birds of the 3rd group were used as control non treated group. Blood samples were collected from ten birds of each group at 25 and 35 days of the experiment. Number of dead birds were recorded daily, body weight and feed conversion were estimated weekly till the end of the experiment (at 45 day). Also, the lymphoid organs (thymus, bursa and spleen) were carefully separated & weighed and each organ relative weight was determined. It was observed that administration of gentamicin (10mg/kg)did not induce any significant changes of blood cell count and relative weights of the lymphoid organs. Additionally, it decreased the phagocytic activity, serum total protein and albumin upto 1 week post vaccination. On the other hand, administration of gentamicin (20mg/kg) decrease the blood cell count, phagocytic activity and the relative weights of the bursa. Both doses of gentamicin provoked a significant decrease in serum globulin, HI titer and protection %.

INTRODUCTION

Vaccination is very important for prevention of many diseases. The failure of vaccination is a real problem and can be affected by several factors as pollution, chemicals, infectious agents, hormones and nutrition . Moreover, chemotherapeutic agents play a critical role in stimulation or suppression of the immune response. Under field condition ,it is unusual to run a commercial poultry cycle without some medication as antibiotics & anticoccidials .

Aminoglycosides, such as gentamicin, are a class of clinically important antibiotics used extensively in the treatment of bacterial infections, particularly against aerobic Gram negative bacteria ***(Nagai and Takano 2004).*** After oral administration, gentamicin is not very effective because it is not absorbed to an appreciable extent from the intestinal tract. The recommended routes of administration of gentamicin are intravenous, intramuscular, subcutaneous ,intraperitoneal or topical. All aminoglycosides can cause varying degrees of ototoxicity ,nephrotoxicity and hepatotoxicity ***(Ali and Goetz.1997;Islam et al., 2011; Giguère et al., 2013*** & ***Elyazji and Abdel-Aziz 2013).*** Injections of gentamicin at a dose of 10mg/kg b. wt are safe ***(Khan et al .,2008)***.

A large number of antibiotics appear to have immunomodulating effects on the animal ***(Yourtee and Root,1984).*** The concept of immunostimulating effect relates to non – specific activation of the immune system implying a non – antigen dependent stimulation of the function and efficiency of macrophages, lymphocytes …etc. Being non

specific, it is expected to give protection against different pathogens including viruses, bacteria and fungi and constitutes an alternative or adjunct to conventional chemotherapy.

Some antibiotics have been found to suppressed the immune response particularly ampicilline ***(Miyata et al., 1998)***, tylosin ***(Abdel-fattah et al., 2004),*** tetracycline ***(Van-Valem et al., 1996)***, sulphadimidine***, (Al-ankari et al., 1996)*** and chloramphinicol ***(Rzedzicki et al., 1991).*** Other antibiotics work to strength the immune response of the animal as enreofloxacin ***(Abdel-fattah et al., 2004),*** macrolide ***(Martin et al., 1980 &Shryock et al., 1998)*** and flumequine ***(Mansur-ud-Din et al .,2007).***

The present work was designed to shed some light on the immunomodulating effects of gentamicin on broilers vaccinated with Newcastle Disease virus.

MATERIALS AND METHODS

Drug:

Gentamicin(Ato-Gent)was purchased from Atco –Pharma**.**

Newcastle Challenge strain:

A local velogenic viscerotropic strain of New castle Disease Virus(NDV) was obtained from the Veterinary institute for biological products and vaccines (Abbasia,Cairo).

Chickens:

Ninety, one day old Hubbard chicks were used in the present study. The chicks were floor reared in experimental rooms bedded by chaffed

wood and provided with clean feeders and drinkers. They were fed a balanced commercial poultry ration. No drugs or vaccines were given to the chickens along the coarse of experiment except those under investigation.

Experimental design:

The chicks were divided into three equal groups, each of 30 chick. The 1st group received 10 mg gentamicin /kg b. wt S/C at 14th, 15th and 16th days of age. The 2nd group received 20 mg gentamicin /kg b. wt S/C at the same period. The 3rd group was left as a vaccinated control group without drug treatment. All birds were given Newcastle disease virus vaccine (Lasota) at 18th day of age.

All groups were challenged with a virulent strain of ND at the30th day of age. At 25 and 35 day of the experiment, blood samples were collected from ten birds of each group. The experimental period lasted for 45 days. The live body weight of all birds and the total amount of feed intake were recorded weekly. The final live body weight was obtained and the feed conversion was calculated (feed intake / weight gain). Number of dead birds were also recorded throughout the experiment. Protection % was recorded for each group (number of survived birds / number of challenged birds). Birds were sacrificed at 45 days then the lymphoid organs (thymus, bursa and spleen) were carefully separated &weighed and each organ relative weight was determined.

Laboratory examinations:

Phagocytic activity was estimated according to ***Barry et al. (1989).*** Total erythrocytic and leukocytic counts were evaluated according to

Natt and Herrick, (1952). Blood film was prepared and stained with Giemsa stain for differential leukocytic count according to ***Schalm et al., (1975).***

Determination of serum total protein was performed according to ***Doumas et. al. (1981)***. Fractions of serum proteins were detected by using polyacrylamid gel electrophoresis as described by ***Laemmli(1970).***

Statistical analysis:

All obtained data were recorded and analyzed statistically by ***Gomez and Gomez, (1984).***

RESULTS

The obtained data presented in tables from (1) to (6) showed that the administration of gentamicin (10mg/kg) did not induce any significant changes of blood cell count and relative weights of the lymphoid organs. Also, it decreased the phagocytic activity, serum total protein and albumin upto 1 week post vaccination. On the other hand, administration of gentamicin (20mg/kg) decrease the number of blood cell count, phagocytic activity and the relative weights of the bursa. Additionally, both doses of gentamicin provoked a significant decrease in HI titer and serum gammaglobulin. Moreover, challenge of treated chickens with Newcastle disease virus resulted in 83% and 70% protection for recommended and double doses of gentamicin respectively compared with 90% protection for vaccinated non treated bird .

Table (1): Effect of gentamicin administration on the total serum protein, albumin and globulin(g/dL).

Age	Treatments	Total serum protein	Serum albumin	Serum globulin		
				Alpha globulin	Beta globulin	Gammaglobulin
25 day	Gentamicin (10 mg/kg)	4.29±0.26^{b}	2.23±0.13^{b}	0.6.9 ± 0.41^{b}	0.87±0.74^{b}	0.50 ± 0.22^{b}
	Gentamicin (20 mg/kg)	3.61±0.18^{c}	1.89±0.96^{c}	0.61 ± 0.3^{c}	0.72±0.31^{c}	0.39 ± 0.31^{c}
	Control	4.73±0.42^{a}	2.41±0.24^{a}	0.77 ± 0.53^{a}	0..96± 0.81^{a}	0.58 ± 0.44^{a}
35 day	Gentamicin (10 mg/kg)	4.30±0.65^{a}	2.36±0.21^{a}	0.79 ± 0.46^{a}	0.85± 0.86^{b}	0.30± 0.42^{b}
	Gentamicin (20 mg/kg)	3.41±0.18^{b}	1.84±0.91^{b}	0.65 ± 0.29^{b}	0.73±0.61^{c}	0.19 ± 0.31^{c}
	Control	4.42±0.33^{a}	2.33±0.16^{a}	0.75 ± 0.43^{a}	0.97±0.78^{a}	0.37± 0.23^{a}

Means followed by a common letter are not significantly different at 5% level by DMRT.

Table (2): Effect of gentamicin administration on blood cell count, phagocytic activity and (HI) antibody titer (log 2).

Age	Treatment	Total RBcs count (10^{6}/µl)	Total leukocytic count (X 1000 cells / cmm)	phagocytic activity	HI titer
25 day	Gentamicin (10 mg/kg)	3.27 ± 0.01^{a}	38.3±3.2^{a}	22.15+ 0.64^{b}	5.0 ± 0.54^{b}
	Gentamicin (20 mg/kg)	2.42 ± 0.02^{b}	29.6±1.8^{b}	21.24+ 0.24^{b}	4.3 ± 0.43^{c}
	Control	3.36 ± 0.02^{a}	39.0±2.6^{a}	24.38+ 0.64^{a}	6.5 ± 0.58^{a}
35 day	Gentamicin (10 mg/kg)	3.31 ± 0.32^{a}	38.8±4.1^{a}	22.74 ± 0.58^{a}	5.5±0.61^{b}
	Gentamicin (20 mg/kg)	2.56 ± 0.29^{b}	29.2±2.4^{b}	20.67± 0.58^{b}	4.5±52^{c}
	Control	3.54 ± 0.38^{a}	39.3±4.7^{a}	23.33 ± 0.58^{a}	6.8±0.41^{a}

Means followed by a common letter are not significantly different at 5% level by DMRT.

Table (3): Effect of gentamicin administration on differential leukocytic count

Age	*Parameters* / *Treatments*	Blood monocytes(%)	Blood lymphocytes(%)	Blood heterophils(%)	Blood esinophils(%)	Blood basiophils (%)
25 day	Gentamicin (10 mg/kg)	9.7 ns ±0.36	63.0 ns ± 1.14	23.6 ns ± 0.56	2.4 ns ± 0.45	1.3 ns ± 0.37
	Gentamicin (20 mg/kg)	10.10 ns ±0.43	62.6 ns ±o.82	23.4 ns ±0.45	2.5 ns ± 0.23	1.4 ns ± 0.48
	Control	9.4 ±0.25	65.1 ±1.32	21.7 ± 0.83	2.4 ± 0.43	1.4 ± 0.37
35 day	Gentamicin (10 mg/kg)	11.8 ns ±0.50	60.6 ns ±1.03	24.5 ns ±5.97	1.3 ns ±0..32	1.8 ns ±0.53
	Gentamicin (20 mg/kg)	11.7 ns ±0.48	59.5 ns ±0.92	26.2 ns ±3.81	1.3 ns ±0.31	1.3 ns ±0.24
	Control	11.4±0.42	61.7±1.1o	23.7±9.o3	1.5±0.41	1.7±0.5

Ns = non significant

Table (4): Live body weight in grams in chickens throughout the experimental period.

Age / Treatment	O day	Ist week	2nd week	3rd week	4th week	5th week	6th week	Final body weight
Gentamicin (10 mg/kg) at 14th,15th and 16th day	40.9±1.1	138±7.2	347±32.4	542± 18.7	997±19.2^{b}	1412±31.8^{b}	1808±25.6^{b}	1935±37.8^{b}
Gentamicin (20 mg/kg) at 14th,15th and 16th day	42.1 ± 1.2	140±9.5	365±25.7	564±23.4	962±21.4^{b}	1388±23.4^{b}	1663±24.3^{c}	1818±41.1^{c}
Control	**39.8±1.4**	**132±6.6**	**355±24.5**	**587±19.7**	**1123±29.8^{a}**	**1568±39..5^{a}**	**1963±21.5^{a}**	**2148±32.1^{a}**

Means followed by a common letter are not significantly different at 5% level by DMRT.

Table (5): Effect of gentamicin administration on feed conversion and protection percentage against challenge with a virulent Newcastle disease virus in broiler chickens vaccinated with NDV.

Treatment	Feed conversion	protection percentage
Gentamicin (10 mg/kg)	2.05±0.024[c]	%83
Gentamicin (20 mg/kg)	2.19±0.048[b]	%70
Control	**1.83±0.089[a]**	**%90**

Means followed by a common letter are not significantly different at 5%level by DMRT.

Table (6): Effect of gentamicin administration on the relative weight of bursa, spleen and thymus.

Parameters / Treatments	Bursa relative weight	Spleen relative weight	Thymus relative weight
Gentamicin(10 mg/kg)	2.125± 0.165[a]	1.782± 0.136	2.211 ± 0.183
Gentamicin (20 mg/kg)	1.525± .231[b]	1.768 ± 0.126	2.136 ± 0.237
Control	**2.342± 0.152[a]**	**1.811 ± 0.142**	**2.245 ± 0.190**

Means followed by a common letter are not significantly different at 5% level by DMRT.

DISCUSSION

The obtained data revealed that gentamicin mainly at a dose of 20 mg/kg significantly suppressed the immune response of birds where it decreased serum total proteins, relative weight of bursa, phagocytic activity, HI titer and protection percentage . The fore mentioned finding fits neatly with those narrated by ***Arivuchelvan et al., (2012)*.** The authors reported that gentamicin produced significant reduction in serum total protein, albumin and globulin beside numerical decrease in HI titer against Newcastle vaccine. Also, ***Saleemi et al., (2009)*** reported that

serum total proteins ,albumin and weight gain decreased while creatinine and ALT increased in chicks given 20 mg/kg and higher doses of gentamicin. The no observable effect level (NOEL) of a single intramuscular administration of gentamicin in day-old broiler chicks was 10 mg/kg body weight .The reduction in serum total protein might be due to inhibition of protein synthesis by gentamicin ***(Buss et al.,1985).***

On the other hand, ***Asha et al. (1999)*** mentioned that gentamicin at levels of 4 mg/kg and 8 mg/kg b. wt doses in rabbits had no significant effect on the immune response and this may be due to species variation beside using low doses of gentamicin.

The data in table (1) & (2) cleared that both doses of gentamicin elicited a significant decreases in HI antibody titer and serum gammaglobulin. These results are in accordance with ***Naqi et al., (1984)*** who mentioned that gentamicin decreases all serum immunoglobulins fractions in chickens. On similar grounds***, Sakaeva and Lazareva (1998)*** found out that administration of gentamicin deepens the severe suppression of antibody genesis against the background of cyclophosphan in mice.

The decreased antibody titer in chicks given gentamicin may be due to the decreased number of leukocytes especially lymphocytes. The decreased gamma globulin level, which is the main source of antibodies synthesis could be another explanation ***(Coles, 1986).*** It is well known that B.lymphocytes developed in the bursa of Fabricius .It play an important role in antibody production ***(Tizard, 2000)*** so ability of gentamicin (20mg/kg) to induce a significant decrease in bursa / body weight ratio may be responsible for the decreases of antibody titer

recorded in this study. The low number of blood cell in gentamicin (20mg/kg) treated group (table 2) may be due to inhibition of protein synthesis which is important for cell mitosis ***(Laurence and Bennet, 1985).***

The present results demonstrated that gentamicin (20 mg /kg) significantly decreased the phagocytic activity (table2). The findings reported by ***Ziv, et al., (1983)*** are in agreement with the present result. The authors recorded that gentamicin had adverse effects on phagocytic capability of cattle phagocytes. The ability of gentamicin to decrease phagocytic capacity may be through alteration of phagocytic cell surface. This explanation was confirmed by ***Paape et al., (1990)*** who observed that gentamicin decreased the phagocytosis through alteration of polymorphnuclear leukocytes morphology.

In conclusion, the present study showed that gentamicin had a dose dependant immunosuppressive effect on broilers. In accordance and to have efficient vaccination, higher doses of gentamicin should be avoided.

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دراسة علي التأثير المناعي للجنتامايسين على الدجاج المحصن بلقاح نيوكاسل

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استهدف هذا البحث دراسة تأثيرات استخدام الجنتامايسين على الاستجابة المناعية لدجاج التسمين المحصن بلقاح النيوكاسل. قسمت الكتاكيت إلى ثلاث مجموعات متساوية كل منها تحتوى على 30 كتكوت المجموعة الأولى تم حقنها بالجنتامايسين (10 mg/kg). في اليوم 14و15و16 بينما المجموعة الثانية فقد حقنها بالجنتامايسين (20mg/kg) فى نفس المدة أما المجموعة الأخيرة فكانت مجموعه ضابطه.تم تحصين جميع الطيور بلقاح لاسوتا في اليوم 18 وعند اليوم 30 تم العدوى بفيروس النيوكاسل.عند يوم 25 و 35 من بداية التجربة تم اخذ عينات الدم من الطيور في كل المجموعات لإجراء عدد من الفحوص المختلفة. معدل التحويل الغذائي ونسبه النفوق تم تسجيلهم بنهاية التجربة عند عمر 45 يوم وكذلك تم حساب الوزن النسبي للغدة الزعتريه وغدة البرسافابريش والطحال في كل مجموعه.

وقد اتضح من هذه الدراسة أن استخدام العقار بكلى الجرعتين السابقتين أدى إلى نقص ملموس في مستوى الجلوبيولين والأجسام المناعية لمنع التلازن الدموي ضد فيروس النيوكاسل. لم يحدث أي تأثير ملموس على العدد الكلى لخلايا الدم الحمراء والبيضاء ووزن الأعضاء الليمفاوية عند استخدام الجرعة الأولى من العقار بينما أحدثت الجرعة المضاعفة نقصا ملموسا في الوزن النسبي لغدة البرسافابريش والعدد الكلى لخلايا الدم الحمراء والبيضاء والنشاط البلعمي. على الجانب الآخر وصلت نسبه الحماية إلى 83% في المجموعة الأولى عند العدوى المباشرة بفيروس النيوكاسل وانخفضت إلى 70% في المجموعة الثانية بينما وصلت إلى 90% في المجموعة الضابطة.