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THE ROLE OF CERTAIN BIOLOGICAL PRODUCTS IN CONTROL OF CHICKEN COLIBACILLOSIS

(With 4 Tables and 3 Figures)

By

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(Received at 21/3/2005)

دور بعض المستحضرات البيولوجية فى مقاومة عدوى الميكروب القولونى فى الدجاج

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تمت فى هذه الدراسة تقييم المعالجة الوقائية والعلاجية للأجسام المضادة الغير نوعية المحضرة من سيرم العجول والدواجن ضد عدوى الميكروب القولونى فى الدجاج. وكانت نتيجة التقييم أن الأجسام المضادة المحضرة من سيرم العجول (آى جى جى) هو أفضل الأجسام فى المعالجة الوقائية والعلاجية ويلية الأجسام المضادة المحضرة من سيرم الدجاج (آى جى واى) بينما الأجسام المضادة المحضرة من سيرم الدجاج (آى جى إم) الأفضل فى المعالجة الوقائية.

SUMMARY

In the present investigation, non-specific immunoglobulins rich-fraction including; bovine-IgG, avian –IgY, bovine IgM and avian IgM were evaluated for prophylaxis and therapeutic effect aganist chicken colibacillosis. The obtained results revealed that bovine IgG rich-fraction was the best one in the prophylaxis and therapy of chicken colibacillosis followed by avian IgY rich-fraction while avian IgM- rich-fraction was the best in prophylactic effect.

Key words: *Chicken colibacillosis, immunoglobulins, E.Coli*

INTRODUCTION

E. coli infections has been increasingly detected among poultry flocks. The pathogenicity of chicken colibacillosis begins as a respiratory infection then invades the blood stream leading to infection

of the deeper organs (liver, heart, oviduct and peritonium) resulting in large economic losses (Vandemaele *et al.* 2002). Moreover; *E. coli* isolated from chickens has a zoonotic significance where Saad *et al.* (1974) proved their pathogenicity to infants. Chemotherapy have been widely used for the treatment and control of *E. coli* respiratory infections in poultry flocks (Dhillon and Jack 1996 and Goren *et al.* 1982) however; the emergence of drug resistance (Allan *et al.* 1993, Dhillon and Jack 1996 and Papadopoulou *et al.* 1997) and their use for prolonged time is likely to induce mutant antibiotic resistance strains (Ginnus *et al.* 1996). Moreover; certain antibiotics are immunosuppressive for broilers (Rzedzicki *et al.* 1991). The costs associated with administration of drugs as well as the effect of drug residues on public health status are factors which increased the interest in usage of an alternative methods for protecting poultry flocks against such infections. Serum immunoglobulin (Ig) rich fractions prepared from pooled bovine or chicken blood had been used in prevention of experimentally induced colisepticaemia (Penhale *et al.* 1971; Logan and Penhale 1971b; Awaad 1975; Kutkat 1988 and Cadman *et al.* 1994). The present study was conducted to evaluate the potential use of Igs fractions derived from bovine or chicken sera as seroprophylaxis or therapeutic treatment for colibacillosis in broiler chickens.

MATERIALS and METHODS

Chicks: One-day old broiler chicks (Cobb) obtained from a commercial source and from nonvaccinated dams against *E. coli* maintained and deep litter system were used. Water and feed supply were added ad libitum. No vaccines were administered against any poultry diseases during the experimental period

Escherichia coli strain: ***(E. coli).*** *E. coli* strain isolated from chicks suffering from colisepticaemia was used. Identification and purification of the isolate was carried out according to Edwards and Ewing (1972), and the isolate was serotyped as O1 with locally prepared sera from standard *E. coli* O1: K1 antigen according to Sojka (1965) and Hassanein (1977).

Bacteriology: Before *E. coli* inoculation, 10 chicks were taken at random, sacrificed and subjected to bacterial examination which proved to be free from any *E. coli* infection as well as organs from dead and sacrificed birds were examined bacteriologically to reisolate the inoculated *E. coli* strain.

Serology: Serum samples of sacrificed chicks at 1, 3, 9, 13 and 21 days of age were examined against *Mycoplasma gallisepticum* antigen by SPA – test using MG stained antigen.

Preparation of gamma globulins rich fraction from calf and chicken: (According to Awaad; 1975 and Shepherd and Dean; 2000):

a) Serum samples: Samples were taken from chicken and calf slaughter houses and concentrated using saturated solution of ammonium sulphate (40% and 25%) to precipitate Igs.

b) Fractionation of calf and chicken IgG (IgY) rich fraction:

- The required volume of 80% saturated solution of amm. sulphate was prepared.
- The volume of serum to be fractionated was placed in a beaker to which slowly added equal volume of 80 % amm. sulphate solution while gently stirred. The mixture was left for 4 h. at room temp. the precipitated globuline was collected by centrifugation at 6000 r.p.m. for 30 min. in cooling centnifige.The supernatant fluid was decanted. The precipitate was resuspended in distilled water to the original volume of serum. The precipitation was repeated as outlined before. The precipitated globulin was resuspended in half the original volume of serum.

The globulin solution was placed in a dialysis bag of visking tubing (27/ 32 size) and amm. sulphate was removed by dialyzing at 4°C against frequent change of 0.85 % Na Cl pH 8.0, until barium chloride test gave negative result.

The globulin sol. was removed from the dialysis bag and sterilized by filtration (0.45 um swirex filter).

c) Fractionation of calf and chicken IgM rich fraction:

The same procedure was applied as done before in IgY separation but using 50 % saturated amm. Sulphate.

Experimental design:

Three experiments were carried out in this investigation as shown in Table (1):

Exp. 1: Studying the prophylactic effect of Igs. against *E. coli* infection in 2-day-old chicks:

Seventy, 2-day-old chickens were divided into 5 equal groups (A, B, C, D and E) consisting of 14 chicks each . Groups A, B, C and D were injected subcutaneously with 0.5 ml. of bovine IgM, avian IgM, bovine IgG or avian IgY respectively. Chicken group E remained without gammaglobulin inoculation as control. Twenty-four hours, after gammaglobulin rich fractions inoculation, all groups were subdivided

into 2 equal subgroups (1 and 2) and were challenged with *E. coli* via oral (subgroups 1) or S/C (subgroups 2) routes of inoculation in a dose of 0.5 ml. (7.5 X 10^6 CFU) and 0.2 ml.(3 X 10^6 CFU) per chick respectively. Chicks of the oral route administration were challenged two successive days.

Exp. 2: Studying the prophylactic effect of Igs. against *E. coli* infection in 8-day-old chicks:

One-hundred, 2-day-old chickens were divided into 5 equal groups (1, 2, 3, 4 and 5) consisting of 20 chicks each. Groups 1, 2, 3 and 4 were injected subcutaneously with 0.5 ml. of bovine IgM, avian IgM, bovine IgG or avian IgY respectively. Group 5 remained without gammaglobulin injection. Eight-day, after gammaglobulin rich fractions inoculation, all groups were subdivided into 2 subgroups (a and b) consisted of 10 chicks each. All subgroups were challenged with *E. coli* via oral (subgroups 1) and S/C (subgroups 2) routes of inoculation in dose of 0.5 ml. (7.5 X 10^6 CFU) and 0.3 ml. (4.5 X 10^6 CFU) per chick respectively. Chicks of the oral route administration were challenged two successive days.

Exp. 3: Studying the therapeutic effect of Igs. against *E. coli* infection in chicks:

Forty, 8-days-old chicks were divided into 5 equal groups (I, II, III, IV and V) consisting of 8 chicks each. All groups were S/C inoculated with *E. coli* in dose of 0.3 ml. per chick (4.5 X 10^6 CFU). After appearance of 1st. clinical signs (3 days post infection), chicken groups I, II, III and IV were subcutaneously inoculated with 0.5 ml. bovine IgM, avian IgM, bovine IgG or avian IgY respectively. Group (V) remained without treatment as control.

All experimental birds were kept for 21 days post infection with *E. coli* under observation for recording symptoms and mortalities. Dead as well as survived chicks were exposed to post mortem and bacteriological examinations.

RESULTS

Obtained results are shown in Tables (2 – 4) and Figs. (1 – 3).

Symptoms: One-day after S/C and four-days after oral inoculation with *E. coli*, some chicks developed: depression, drooping wings, sleepy appearance, inappitence, staggering gait, dullness, hiding together and respiratory distress. Seven days post infection, some chicks developed pasty vents recumbency and rales sound as respiratory symptoms.

Post mortem lesions: Chicks that died within the observation period showed the following lesions: After 2 days, showed fibrenous pericarditis, perihepatitis, unabsorbed yolk sac, caseous yolk sac contents and enlarged gall bladder. After first week, showed thickened air sac containing yellow caseous material, fibrenous pericarditis, fibrenous perihepatitis and generalized visceral congestion. Survived chicks were sacrificed at the end of observation period and showed no characterized lesions except slight air saculitis for chicks S/C inoculated with *E. coli*.

Bacteriological examination:

All chicks which died within the period of observation were positive for reisolation of *E. coli* strain either S/C. or orally inoculated. Chicks sacrificed at the end of observation period were negative for bacterial reisolation for the inoculated *E. coli* strain.

DISCUSSION

Poultry constitutes a significant sector of the world economy. The intensive production of broiler chickens are the largest segment of poultry industry. *E. coli* is a major pathogen of the world wide importance in commercially produced poultry and produced heavy economic losses among baby chickens (Gross, (1964) and Awad *et al.* (1973). Since, Smith and Littlie (1922) first suggested that bovine serum might be used as an alternative to colostrums to protect the newly born calf against colibacillosis.

Relatively few studies had been developed in study the potential use of immunoglobulins for controlling chicken colisepticemia (Awaad, 1975, Youssef, 1976 and Kutkat, 1988). Penhale, (1965 a and b) mentioned that the major immune component present in normal bovine serum having antibacterial activity, particularly against Gram negative bacteria including pathogenic serotypes of *E. coli,* is immunoglobulin M (IgM).

The results of our investigation study of non-specific gammaglobulins in chickens challenged with *E. coli* (Table 2 and Fig.1) showed that bovine IgG gave 85.7 % and 71.3 % protection for chickens challenged orally or S/C respectively followed by administration of avian IgM that gave 57.1 % and 42.9 % protection for chickens challenged orally or S/C respectively. These results accord witrh those described by Logan *et al.* (1974) who reported that all three immunoglobulins classes of bovin serum (IgA, IgG and IgM) appear to

be involved in the provision of immunity to the enteric syndrome. However, IgA was the least effective the IgG or IgM appeared to be relatively more important. Moreover; our results are in agreement with that obtained by Awaad (1975) who found that inoculated intramuscular gamma-bov and non specific gamma-av IgM rich-fraction gave best results of protection (100% and 60%) respectively in prophylactic effect of gammaglobulins in chickens after challenged with pathogenic *E. coli* S/C. Also, our results are completely accord with those obtained by Youssef (1976) in turkeys inoculated I.M. with bovine immunoglobulins and Kutkat (1988) in chickens inoculated I.M. with IgM-av rich fraction.

The obtained results in (Table 3 and Fig. 2) showed the best results of protection with prophylactic dose (0.5 ml. S/C) of avian IgM, bovine IgG and avian IgY for 2-days-old chicks in a ratio of (100, 80 and 80%) and (80, 80 and 60%) for E. coli inoculated at 8 days-old orally or S/C respectively. Our results are in complete agreement with those obtained by Awaad (1975) who studied the therapeutic effect of gamma globulins inoculated intramuscularly in chickens infected with *E. coli* and found that gamma-bov (bovis) and specific gamma-av (avian IgY) rich-fractions and avian IgM gave best results, while nonspecific gamma-av. gave 60% and 40% for IgM and IgY respectively. The obtained results are in accordance with the obtained results by Kutkat (1988) who I.M. inoculated chickens with IgM-av rich fraction. On the contrary, Youssef (1976) in turkeys I.M. inoculated IgM-av and IgY-av rich fraction and gave low results of protection on studying the prophylactic effect.

The evaluation of the results obtained from the therapeutic effect of gammaglobulins treated in chickens infected with *E. coli* then treated the symptoms with non-specific immunoglobulins 3 days later (Table 4 and Fig. 3) revealed that the best results were obtained from the inoculation of 0.5 ml. bovine IgG or bovine IgM with protection of 87.5% for each. While avian IgY rich-fraction gave 75% protection with the same dose. These results confirm the conclusion mentioned by Awaad (1975) who fonud the best results obtained by intramuscular inoculation of gamma-bov (100%) and disagree with that obtained by intramuscular inoculation of non-specific gamma-av IgY or IgM (40% and 60%) in controlling experimentally induced chicken colisepticemia. On the other hand; these results disagree with those obtained by Kutkat (1988) who found that IgM-av gave low percentage of protection on I.M. and oral inoculations as 35.3 and 27.8 % with *E. coli* O78: K 80 while with *E. coli* O125: K 70 were 10 and 9 % respectively. On the

other hand, Rainard (1986) carried in vitro a study on *E. coli* strain B 117 and found that the bovin immunoglobulins did not influence multiplication of *E. coli*.

The role of gammaglobulins in protection against *E. coli* infection recorded in the present investigation have been previously reported in piglets by Corbella (1970), in calves by Walser and Brumwer (1967); Draghici *et al.* (1969); Penhale *et al.* (1971); Logan *et al.* (1974) and in chickens by Awaad (1975).

In conclusion; bovine IgG rich-fraction gave the best results in prophylaxis and treatment chicken colibacillosis followed by avian IgY rich-fraction and avian IgM rich-fraction in prophylactic effect.

ACKNOWLEDGMENT

We are grateful to Prof. Dr. Hassanin, Z.A.W. (Poultry Dis. Dept.; AHRI.) for his providing the culture of *E. coli* isolate. and technical support in the present study.

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Table 1: Experimental design for administration of non-specific immunoglobulins rich fraction as prophylactic and therapeutic treatment in chickens challenged with *E. coli.*

	Group No.	Chick No.	Ig.	Treated Ig time	Sub-group.	Chick No. per SubGr.	Infected Route	Infected Time of infection
Exp. No. 1	A	14	IgM-bov	2-day-old	A1	7	Oral	3-day-old
					A2	7	S/C	
	B	14	IgM-av		B1	7	Oral	
					B2	7	S/C	
	C	14	IgG-bov		C1	7	Oral	
					C2	7	S/C	
	D	14	IgY-av		D1	7	Oral	
					D2	7	S/C	
	E	14	-	-	E1	7	Oral	
					E2	7	S/C	
Exp. No. 2	1	20	IgM-bov	2-day-old	1a	10	Oral	8-day-old
					1b	10	S/C	
	2	20	IgM-av		2a	10	Oral	
					2b	10	S/C	
	3	20	IgG-bov		3a	10	Oral	
					3b	10	S/C	
	4	20	IgY-av		4a	10	Oral	
					4b	10	S/C	
	5	20	-	-	5a	10	Oral	
					5b	10	S/C	
Exp. No. 3	I	8	IgM-bov	11-day-old		-	S/C	8-day-old
	II	8	IgM-av			-	S/C	
	III	8	IgG-bov			-	S/C	
	IV	8	IgY-av			-	S/C	
	V	8	-	-		-	S/C	
Cont. – ve.*		10 + 10	-	-	-	-	-	-

* Control - ve (10 + 10) chicks = 10 chicks for exp. 1 + 10 chicks for exp. 2 and 3.

Table 2: The prophylactic effect of subcutaneous treatment of bovine and avian Igs. rich fractions inoculated subcutaneously at 2-days-old for chickens and challenged with *E. coli* subcutaneously and orally after 24 hours.

Gr. No.	Subgroup No.	Chicks No.	Ig	Rout of challenge	Mortalities / week			Total mortalities		Total survival	
					1 st.	2 nd.	3 rd.	No.	%	No.	%
A	A1	7	IgM– bov.	Oral	2	-	-	2	28.6	5	71.4
	A2	7		S/c	5	2	-	7	100	0	0
B	B1	7	IgM – av.	Oral	2	1	-	3	42.9	4	57.1
	B2	7		S/c	4	-	-	4	57.1	3	42.9
C	C1	7	IgG– bov.	Oral	1	-	-	1	14.3	6	85.7
	C2	7		S/c	1	1	-	2	28.6	5	71.4
D	D1	7	IgY – av.	Oral	5	-	-	5	71.4	2	28.6
	D2	7		S/c	5	-	-	5	71.4	2	28.6
E	E1	7	-	Oral	4	1	-	5	71.4	2	28.6
	E2	7		S/c	5	1	-	6	85.7	1	14.3

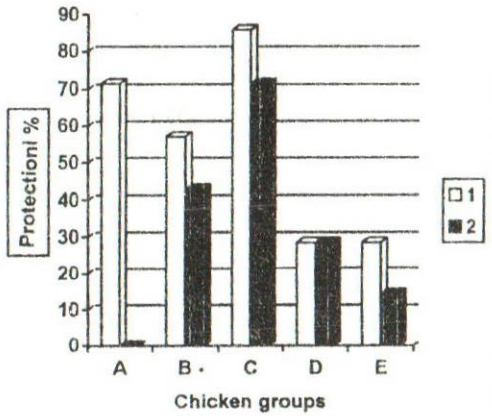
Table 3: The prophylactic effect of subcutaneous treatment of bovine and avian Igs. rich fractions at 2-days-old for chickens challenged with *E. coli* subcutaneously at 8-days-old.

Gr. No.	Subgroup No.	Chicks No.	Ig	Rout of inoculation	Mortalities / week			Total mortalities		Total survival	
					1 st.	2 nd.	3 rd.	No.	%	No.	%
1	1a	10	IgM– bov.	Oral	2	2	-	4	40	6	60
	1b	10		S/c	6	2	-	8	80	2	20
2	2a	10	IgM – av.	Oral	0	0	-	0	0	10	100
	2b	10		S/c	2	0	-	2	20	8	80
3	3a	10	IgG– bov.	Oral	2	0	-	2	20	8	80
	3b	10		S/c	2	0	-	2	20	8	80
4	4a	10	IgY – av.	Oral	0	0	-	0	0	10	100
	4b	10		S/c	2	0	-	2	20	8	80
5	5a	10	-	Oral	2	0	-	2	20	8	80
	5b	10		S/c	4	0	-	4	40	6	60

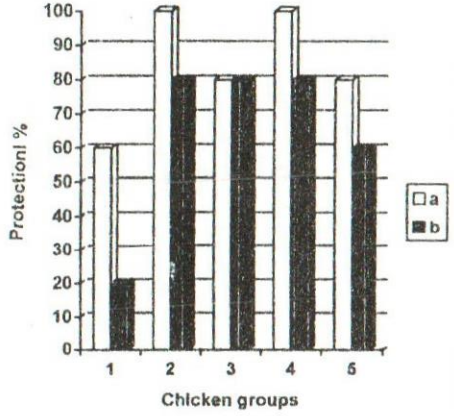
Table 4: The therapeutic effect of subcutaneous treatment of bovine and avian Igs. rich fractions for chickens after showing symptoms of infected with *E. coli* subcutaneously at 8-days-old.

Gr.No.	Chicks No.	Ig	Rout of inoculation	Mortalities / week			Total mortalities		Total survival	
				1 st.	2 nd.	3 rd.	No.	%	No.	%
I	8	IgM – bov.	S/c	1	-	-	1	12.5	7	87.5
II	8	IgM – av.	S/c	2	2	-	4	50	4	50
III	8	IgG – bov.	S/c	1	-	-	1	12.5	7	87.5
IV	8	IgY – av.	S/c	1	1	-	2	25	6	75
V	8	-	S/c	1	1	-	2	25	6	75

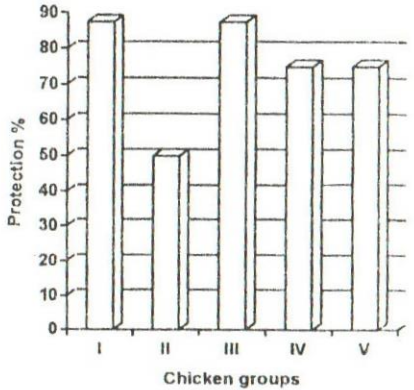
Fig.(1) Protection % of Igs treated chickens (prophylactic study after 24 hs.) against E. coli infection.



Fig(2):Protection % of Igs treated chickens (prophylactic study after 8-days old) against E.coli infection.



Fig(3): Protection % of Igs treated chickens (therapeutic study) against E.coli infection.



1 and a = oral infection with *E. coli.*
2 and b = subcutaneously injection with *E. coli.*
Groups A , 1 and I = inoculated with bovine IgM rich fraction.
Groups B , 2 and II = inoculated with avian IgM rich fraction.
Groups C , 3 and III = inoculated with bovine IgG rich fraction.
Groups D , 4 and IV = inoculated with avian IgY rich fraction.
Groups E , 5 and V = inoculated with *E.coli* only (as control).