Vet.Med.J.,Vol.40,No.3(1992).29:35

THE EFFECT OF PREPARED IMMUNOGLOBULINS RICH FRACTIONS AGAINST EXPERIMENTAL ESCHERICHIA COLI INFECTION IN CHICKENS

BY
M.H. AWAAD* ;A.G. HEGAZI; LAILA, ALI AND M.A. KUTKAT
National Research Center , Dokki , Egypt
Fac. of Vet. Med. ,Cairo University
(Recived 22.10.1992)

INTRODUCTION

Tremendous losses to the poultry industry have been attributed infections with E.coli (**Sojka and Carnaghan, 1961**). Complicating the epidemiology of these infections and increasing their threat to the industry is the evidence indicating their egg born nature (**Panthak et al., 1960 and Awad, 1975**).

From the epidemiological and ecological standpoints of view, these infections are not easy to be controlled. Chemotheraphy in the presence of infectious drug resistance has been looked at as transient and unreliable, besides representing a potential health hazard for human (**Panigrayh et al., 1983**). Owing to the complexity of their antigenic protein makeup and the presence of many different serotypes vaccination against avian colibacillosis seems to be unreliable for practical use (**Sojka, 1965**). Hence the search for other methods of control is commendable.

The optimal ammonium sulphate concentration for fractionation of rabbit, sheep, horse, goat (**Hebert et al., 1973**); bovine and piglets bovine (**Logan et al., 1974**) and piglets (**Kennelly et al., 1979**) were recommended. Three precipitations were needed to free the material from haemoglobulin. Immunoglobulins rich fraction. At the mean time atrials to use them for immunoprophylaxis against E.coli infection in chickens were attempted.

MATERIAL AND METHODS

Preparation of immunoglobulin (Ig)- rich fractions :

a. Determination of the optimal ammonium sulphate concentration for precipitation of IgM and/or IgY:

Pooled chicken blood samples were collected from the processing plant in sterile containers for serum separation. Stock ammonium sulphate saturated solution was pre-

pared according to **Hebert et al., (1973) and Awaad (1975)** and stored at room temperature. Final solutions of 50, 60,70, 80 and 90 percent ammonium sulphate were prepared (V/V) when needed from the stock saturated solution. One volume of chicken serum was gently stirred while an equal volume of ammonium sulphate solutions of a given concentration was slowly added and mixed well to obtain final concentration of 25, 30, 35, 40, 45 and 50 percent of ammonium sulphate serum mixture. The mixtures were left at room temperature for 4 hours and centrifuged to pack the precipitated protein. The supernatant fluids were collected and stored at -20°C. The precipitates were resuspended in distilled water to the original volumes of sera. Two further precipitation cycles were carried out in a similar manner (Higgins, 1975). The fractions concentration was measured by Biuret method (Weichselbaum,

Polyacrylamide gel electrophoresis (PAGE) and immunoelectrophoresis:

The protein fractions was subjected to electrophoretic analysis by using the polyacrylamide gel columns **(Davis, 1964 and Ornstein, 1964)**. The gels were stained with amindo black 12 B in 7% acetic acide then destained. Reading of gels was carried out by scanning through Beckman Scanner **(Harb et al., 1973)**. The conformation, identification and the relative mobility values of the zones were determined as dscribed by **Glick, (1968) and Schellner (1970 a & b)**. The immunoelecterophoretic analysis of the sediments and supernatents were carried out as adopted by **Grabar and Williams (1953)**. The anti-chicken serum was prepared in rabbit according to **Williams and Chase (1968)**.

Experimental:

Prophylactic effect of serum Ig-rich fractions on experimental E.coli-Infection in chickens:

Two-hundered and ten, 6-day-old, male egg-type chickens were divided equally into 10 groups (1-10), housed separately in batteries, and fed on a commercial ration free from antibacterial drugs. Chickens of groups 1-2 were injected with 5.5 and 11.0 mg/bird respectively of IgM-rich fraction intramuscularly, while those of gorups 3-4 were similarly injected with 35 and 70 mg/bird respectively of IgY-rich fraction. Groups 5-6 received 16.5 and 33.0 mg/bird respectively of IgM rich fraction orally, and groups 7-8 received 105 and 210 mg/bird respectively of IgM-rich fraction by the same route. The Ig doses used here in were based on results reported previously by **Awaad (1975)**. Group 9 was kept as infected-nontreated control and group 10 served as noninfected non treated control.

Twenty-four hours after administration of Ig fractions all chickens, except those of gorup 10, were infected subcutaneously with a constant dose of $25x10^6$ viable cells/bird of E.coli serogroup $O_{78}:K_{80}$ (B-) as adopted by **Harry (1964)**. The chickens were kept under observation for 21 days; dead and survivors were subjected to postmortem and bacteriological examinations. In calculating the percentage of protection only those birds which survived at the end of the observation period without evidence of persistence of the inoculated pathogen in their internal organs, as judged by bacteriological examination, were considered according the the following formula:

$$\frac{\text{No. of total survivors-No.of survivors with positive reisolation} \times 100}{\text{Total No. infected}}$$

RESULTS

The optimal concentration of ammonium sulphate for total gammaglobulin precipitation was 30%, while 25% concentration was better than other concentrations in precipitation of IgM. On the other hand, 45% ammonium sulphate concentration was the best for precipitation of IgY (Table 1). The electrophoretic profiles in polyacrylamide gels as well as the immunoelectrophoresis against anti chicken serum were demonstrated in Fig. (1).

Prophylactic effect of serum Ig-rich fractions on experimental E. coli-infection in chickens:

The results are given in Table (2) in infected nontreated birds symptoms appeared 1-3 days and deaths occurred 1-4 days postinfection. In infected Ig-pretreated birds, symptoms and deaths occurred 5-8 days and 10-13 days post infection respectively in orally and intramuscularly pretreated groups. The symptoms observed included depression, somnolescence, huddling, droopy wings and pasty vent.

Mortality in intramuscularly

Table (1) : Recovery of immunoglobulins from pooled chicken sera after precipitation wih different ammonium sulphate concentrations.

Examined sample	Saturated ammonium sulphate %	Protein gm%	Recovered gammaglobulins gm%			Percentage		
			IgM	IgY	Total	IgM	IgY	Total
Original	--	4.7	0.2	1.5	1.7	4.4	32.4	36.8
	25	0.2	0.0	0.1	0.1	11.3	40.0	51.3
The third	30	0.1	0.0	0.1	0.1	5.1	52.1	57.3
pricipitate	35	0.5	0.0	0.3	0.3	6.1	51.1	57.1
by	40	1.1	0.1	0.6	0.6	6.2	50.0	56.2
ammonium	45	2.0	0.1	1.0	1.1	4.5	52.5	57.0
sulphate	50	0.8	0.0	0.3	0.4	5.6	38.7	44.4

Fig. (1): Precipitates obtained from pooled chicken serum with different concentrations of ammonium sulphate.
A: Electrophoretic profiles in polyacrylamide gels.
B: Immunoelectrophoresis.

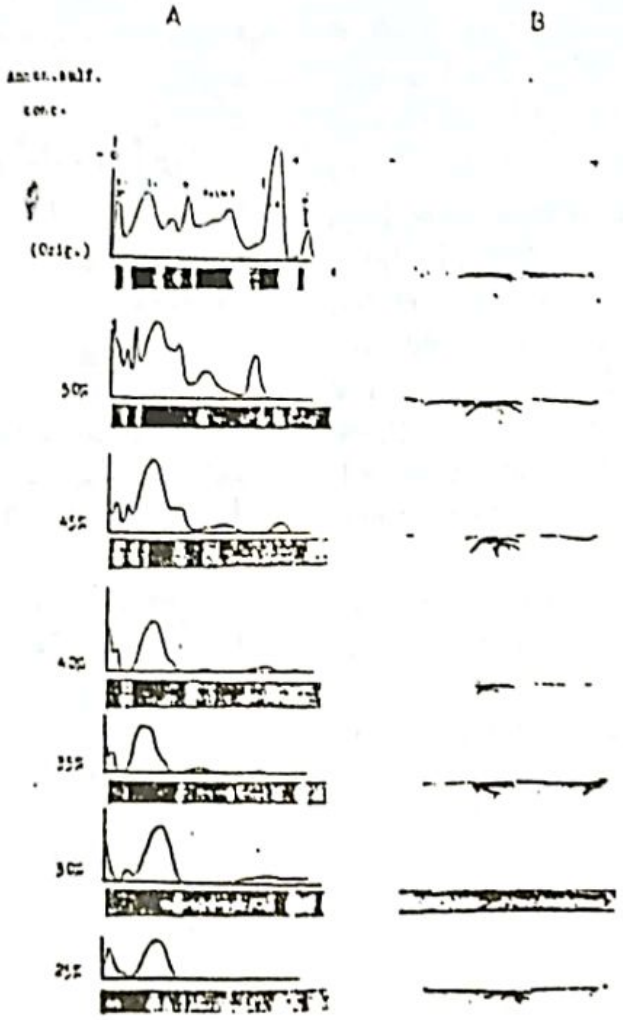

Table (2): Effect of *E.coli* infection (serogroup O_{78}:K_{80} B) on chickens pretreated with serum immunoglobulins 24 hours prior to infection (a)

Group No.	No. of birds / group	Treatment	Rout of Administration	Dose mg/bird	Results of infection						Total Protein (d)	
					Mortality			Survivors				
					No.	%	No. of +ve. reisolations	No	%	No. of +ve. reisolations	No.	%
1	21	IgM	IM	5.5	2	9.5	2	19	90.5	1	8	85.7
2	21	IgM	IM	11.0	1	4.8	1	20	95.2	1	19	90.5
3	21	IgY	IM	35.0	3	14.3	3	18	85.7	4	14	66.7
4	21	IgY	IM	70.0	2	9.5	2	19	90.5	4	15	71.4
5	21	IgM	Oral	16.5	5	23.8	5	16	76.2	3	13	61.9
6	21	IgM	Oral	33.0	2	9.5	2	19	90.5	1	18	85.7
7	21	IgY	Oral	105.0	2	9.5	2	19	90.5	5	14	66.7
8	21	IgY	Oral	210.0	3	14.3	3	18	85.7	5	13	61.9
9 (b)	21	—	—	—	11	52.4	11	10	47.6	6	4	19.0
10 (c)	21	—	—	—		0.0	0	21	100	0	0	100.0

(a) Subcutaneous infection with 25 x 106 viable cells/bird.
(b) Positive control infected nontreated
(c) Negative control (n0uinfected-n0utreated).
(d) Survivor carriers were considered as if dead in calculating the percentage of protection.

pretreated groups ranged between 4.8-23.8% and 9.5-14.3% for IgM and IgY pretreatments versus 9.5-23.8% and 9.5%-14.3% in orally pretreated groups respectively. in infected nontreated birds mortality reached 52.4%

The frequency of reisolation of inoculated E.coli from survivers was generally higher with IgY than with IgM pretreatments regardless of the dose or route of administration while infected nontreated survivors yielded a higher rate of reisolation than either Ig-pretreated gorups. The protection levels against mortality plus persistance of E. coli were comparatively higher with IgM than IgY pretereatments, regardless of the dose or route of Ig-adminstration.

DISCUSSION

The optimal concentration of ammonium sulphate required for precipitation of maximal ammounts of chicken immunoglobulin was investigated. The percentages of recovery of immunoglobulin ranged between 4.5-11.3 and 38.7-52.5 for IgM and IgY rich fractions respectively. These results clearly showed that 30 percent final concntration of ammonium sulphate was superior for precipitaiton of the total immunoglobulin (IgM and IgY), where as 25 and 45 percent final concentrations were optimal for precipitation of maximal amounts of IgM and IgY respectively. Our results completely accord with the confirm those reported by **Awaad (1975)** who recorded the optimal concentrations of the ammonium sulphate for maximal precipitation of chicken immunoglobulin (IgM and IgY). These results might help in the process of producing purified avian immunoglobulins which was considered as a major problem **(Kramer, 1973)** because chicken immunoglobulins precipitate in diluted buffers and polymerize in concentrated ions when separated in ion exchange chromatography.

The low level of IgM obtained in comparison to the IgY level might be explained in the light of the report of **Higgins, (1975)** who concluded that the yields of the fowl IgM in terms of the total quantity of protein are generally low.

The results achieved when IgM or IgY-rich preparation was given 24 hours prior to E.coli (serogroup 78 K_{80}) infection (Table 2) generally indicated that pretreated groups were about 3-5 times more protected against mortality and the inoculated pathogen persisted more than infected nonpretreated groups, regardless of the route of administration or class or dose of Ig used. This confirms previous reports **(Awaad, 1975, Youssef, 1976)** and suggests a considerable protective

value for the Ig preparations used which is presumably of a specific nature. The presence of specific antibody activity to E.coli or other bacterial pathogens in the Ig preparations used was not checked.

In this experiment it was found that the degree of protection achieved was dose, route and Ig-class dependent. Thus, the higher Ig doses were superior to the lower doses, and the intramuscular route and the IgM-rich fraction were more protective than the oral route or the IgY-rich fraction. This influence is presumably due to quantitative difference in antibody content of the inoculum, and differences in the rate of transfer and distribution of the Ig and in the binding capabilities of the Ig molecules.

In conclusion, it appears that the use of serum Ig in the prophylaxis against E. coli infection has a good value in protection of chicken against E. coli infection.

SUMMARY

The optimal concentration of ammonium sulphate required for precipitation of maximal amounts of chicken immunoglobulin (Ig) was determined. The third precipitate and first supernatant fluid obtained from treatement of pooled chicken serum with 25, 30, 35, 40, 45 and 50% final concentrations of ammonium sulphate were electrophoretically analysed in polyacrylamide gels as well as immuno electrophoretically examined against anti-chicken serum prepared in rabbits. 30% ammonium sulphate concentration was optimal for precipitation of total immunoglobulins from chicken serum, 25% for precipitation of IgM and 45% for precipitation of IgY.

When serum IgM or IgY-rich fractio preparation was given 24 hours prior to E coli (serogroup 078:k 80 infection, protected the experimental birds (about 3-: times) against mortality and persistence o the inoculated pathogen than infected nonpretreated groups, regardless of the route of administration or class or dose o Ig used. The degree of protection provec to be dose, route and Ig-class dependent.

REFERENCES

Awaad, M.H. (1975): Studies on E.coli infection in chickens. Ph.D. Vet. (Poultry diseases) Thesis, Cairo Uiv.

Davis, B.J. (1964): Disc electrophoresis, Method and application to human serum proteins. J. Ann. N.Y. Acad. Sci., 121:404-428.

Glick, B. (1968): Serum protein electrophoresis patterns in acrylamide gel/: Patterns from normal and bursaless birds. Poult. Sci., 47: 807-815.

Grabar, P. and Williams, C.A. (1953): A Method permittting the comined study of the electophoretic and immunochemical properties of a mixture of proteins: application to blood serum. Biochem. Biophys. Acta 10: 193-194.

Harb, N.; Hafeez, M.A. and Voaden, D.J. (1973): A regulated temperature disc electrophoresis apparatus. Cited by Awaad, M.H. (1975).

Harry, E.G. (1964): Th survival of E. coli in the dust of poultry houses. Cited by Awaad, M.H. (1975).

Hebert, G.A.; Patricia, L.P. and Bertie, P. (1973): Determination of the opitmal ammonium sulphate concentration for the fractionation of rabbit, sheep, hors and goat antisera. Appl. Microbiol., 25; 26-36.

Higgins, D.A. (1975): Physical and chemical

properties of fowl immunoglobulins. Vet. Bull., 45: 139-154.

Kennelly, J.J.; Ball, R.O. and Aherne, F.X. (1979): Influence of porcine immunoglobulin adminstration on suvival and growth of pigs weaned at two and three weeks of age. Can J. Anim. Sci., 59: 693-698.

Kramer, T.T. (1973): The immunologic response of birds. Avian Dis., 17: 208-213.

Logan, E.F.; Stenhouse, A.; Drmord, D.; Penhale, W.J.; and Mirabelle, A. (1974): Studies on the immunity of the calf to colibacillosis VI. The prophylactic use of a pooled serum IgM rich fraction under field conditions. Vet. Red., 94: 386-389.

Ornstein, L. (1964): Disc electrophoresis, Background and Theory. J.A.nn. N.Y. Acad. Sci., 121:321.

Panigrahy, B., Gyimph, J.E.; Hall, C.F.; and Williams, J.D. (1983): Immunogenic Potency of an oil-emulsified Escherichia coli bacterin. Avian disease 28, 475-481.

Panthak, R.G.; Singh, G.M.; and Tangri, R.P. (1960): Chick mortality and contamination of yolks by members of enterobacteriaceae. Bri. Vet. J. 116:81-84.

Schellner, H.P. (1970 a): Blutbild, Mamatokrit und electrophoretische Auftrennung in polyacrylamidegel von seren gesunder Brieftauben Berf Mun. Tiera Ztl. Wochenschrift, 83: 255-257.

Schellner, H.P. (1970 b): Gesamteiweissbestimmung und elektrophoretische auftrennung in polyacrylamidgel von putenseren. Archiv fur Geflugelkunde, 2: 72-74.

Sojka, W.J.; Carnaghan, R.B.A. (1961): E.coli infection in poultry. Res. Sci., 2: 240-352.

Sojka, W.J. (1965): Escherichia coli in domestic animals and poultry First Ed., Commenwealth agricultural bureaux, Farnhan oyal, Bucks, England, P. 205.

Weichselbaum, T.E. (1946): An accurate and rapid method for determination of proteins in small amount of blood serum and pathol., 16:40.

Williams, C.A. and Chase, M.W. (1968): In: "Methods in immunology and Vol. 1, Second Ed. p. 209-223. Academic Preese, New York.

Yousse f, I.Y. (1976): Studies on E.coli infection in turkeys. Ph.D. Vet. (Poultry diseases). Thesis, Cairo Univ.