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STUDY ON PATHOGENICITY AND IMMUNOGENICITY OF IRRADIATED SPORULATED INTESTINAL EIMERIA OOCYSTS IN CHICKENS

(With 2 Tables)

By

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دراسة التأثيرالامراضى والاستجابة المناعية فى الدجاج عند العدوى بالاكياس المتحوصلة والمشعة لكوكسيديا الامعاء الرفيعة

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عند عدوى الكتاكيت بحويصلات الكوكسيديا المعوية والمعرضة للاشعاع عند جرعة ٢٠ و ٢٢ و ٢٥ كيلو راد لم يتم العثور على الحويصلات فى البراز بدأ من اليوم السادس وحتى اليوم الرابع عشر بينما عثر على الحويصلات بأعداد متفاوتة ومتناسبة عكسيا مع جرعات الاشعاع فى كتاكيت المجموعات المحقونة بحويصلات معرضة للاشعاع عند جرعات اقل من ١٨ كيلو راد كما لوحظ ان معدل الزيادة فى وزن الطيور المحقونة بحوصلات مشعة بجرعات اكبر من ١٠ كيلو راد كانت اكبر بفرق معنوى عنه فى كتاكيت المجموعة التى تم عدوتها بحويصلات غير معرضة للاشعاع . تكرار العدوى بخمسة جرعات متزايدة يوميا بالطور المعدى للكوكسيديا والمعرضة للاشعاع عند جرعات ١٨ و ٢٢ كيلو راد قد اكسبت الدجاج مناعة معنوية ممثلة فى معدل الزيادة فى الوزن ومعدل التغير المرضى فى الامعاء الرفيعة عند اجراء اختبار تحدى المناعة.

SUMMARY

Irradiation of sporulated oocysts of intestinal *Eimeria* reduce their pathogenicity for two-weeks old chickens as determined by weight gains, lesion score and the number of oocysts discharged in droppings. During seven days of infection with 10^5 irradiated sporulated oocysts, the mean weight gains were increased proportionally with the dose of irradiation. The

number of discharged oocysts (6-14 days) post infection as well as lesion score (7th day post infection) was decreased by increasing the dose of irradiation. Repeated inoculation (immunization) of two weeks old chicks with graded doses of irradiated oocysts (18 and 22 K Rad) partially protected chickens against challenge with non-irradiated viable oocysts of intestinal *Eimeria*. After challenge, the mean weight gain of the immunized challenged chickens was significantly higher than unimmunized-challenged group. The lesion scores of the immunized-challenged groups were low compared with the control unimmunized-challenged group.

Key words: *Pathogenicity-Immunogenicity-Intestinal Eimeria in Chickens*

INTRODUCTION

Due to the intense rearing of poultry, coccidiosis is an economically important disease for poultry industry. Coccidiosis is currently controlled by medication, but due to the increasing emergence of drug-resistant strains of coccidia, an alternative control measure have been developed and numerous vaccination strategies have been attempted to control avian coccidiosis. A mixture of different coccidial strains of live parasites has been successfully used in commercial application, but due to potential problems associated with using living parasites, various means of attenuation have been tried by serial embryo passage (Long et al 1982 and McDonald et al 1982), or selection for precocious development (Shirley and Bellatti 1984 and Long and Johnson 1988). Irradiation has been used to attenuate Eimeria parasites (Abu ali et al 1972., Singh and Gill 1975, Hayat 1976, Jungman & Miekel 1989 and Augustine et al.1993). Irradiation dose is important and each Eimeria species show different sensitivity to irradiation dose. An exposure to 20 k.Rad of *E.tenella* oocysts had no effect on their invasive activity, but resulted in reduced merogenic development and immature 1st generation schisonts (Jenkins et al 1991,a&b). Merogenic development was not observed at any time post infection in chickens infected with irradiated *E. maxima* at dose of 12 - 20 k.Rad. (Mark et al 1993). A large scale field trial using radioattenuatted *E.tenella* oocysts in the drinking water of 8-day-old broiler chicken was performed by (Mielke et al 1991). The aim of the present study was to investigate the effect of gamma radiation on pathogenicity of intestinal Eimeria, and to study the immunity induced in chicken administrated graded doses of irradiated oocysts.

MATERIAL and METHODS

Chicks

One day old chicks were obtained from commercial hatcheries reared with an unmedicated commercial feed and water *ad-libitum*.Their faeces were examined periodically by the flotation technique for the presence of coccidial oocysts for two weeks. The birds were randomly distributed to certain treatment groups in a wire floored cages according to the design of the experiments.

Parasites:

Field isolate of Eimeria oocysts were collected from the duodenal contents of chicks showing lesions only in the duodenum. The mucosa showed grayish-white transversely extending superficially situated scale-like lesions and the contents of the bowel appeared as dirty milk. Duodenal contents were suspended in 5% potassium dichromate for 20 minutes then washed 3x with physiological saline and resuspended in 2.5% potassium dichromate for sporultion in 5 ml peter's dishes with intermittent aeration at room temperature. Oocysts were propagated by inoculation of sporulated oocyts via direct injection into the crop of 2 weeks old chicks free from coccidial infection. The oocysts were collected 6 days post infection (P.I.) from the duodenal contents. Sporulated oocysts were washed 2x and resuspended in sterile distilled water for irradiation. Sporulated oocysts were exposed to gamma radiation source (at 110 Rad/sec.) in National Center For Radiation And Research Technology Naser city.

Parameters for evaluating pathogenicity and immunogenicity of irradiated oocysts:

Pathogenicity of irradiated oocysts and their immunity afforded against challenge were evaluated by measuring body weight gain, the mean oocysts output /gram feaces and the lesion scores. Intestinal lesions were graded on a scale between 0 and 4 according to Jhonson and Reid 1970.

Statistical analysis of data.

Treatment groups within experiments were compared statistically using ANOVA (Duncan,s multiple-range) test at 5% level.

Experimental design:-

The effect of gamma radiation on the pathogenicity of *Eimeria* oocyts:

1- Nine groups of 20 birds each, were used. At 21st day of age, chicks of groups 3-9 were inoculated once with 10^5 sporulated oocysts that had been exposed to 5, 10, 15, 18, 20, 22 and 25 k.Rad respectively. Chicks of group 2 were inoculated with 10^5 non irradiated oocysts. Birds of group 1

served as noninoculated control group. Six days post inoculation 10 birds from each group were sacrificed for lesion scoring. The other 10 birds from each group were retained for other 8 days. The faecal materials were collected daily from each group in a separate jar, pooled and the oocysts were counted using McMaster technique (Long et al 1976). All chickens were weight just before inculation and six days post (at time of sacrificing).

studying the immunogenicity of irradiated oocysts:

Four treatment groups and one control group of 20 birds each were used. The chicks were randomly assigned for treatment at 3 weeks. Each chick received 5 graded doses of irradiated oocysts (500, 1000, 2000, 5000, and 10,000 oocysts) daily. Birds of group 3 received irradiated oocysts exposed to gamma radiation at a dose of 18 K.Rad, while the group 4 inoculated with irradiated oocysts at a dose of 22 K.Rad. The viable non irradiated oocysts were administrated to birds of group 5. Chicks of group 2 were inoculated with distilled water. Two weeks after the last inoculated dose, the chicks of groups 2,3,4,5 were challenged with 10^5 viable oocysts. Chicks of group 1 were left as uninoculated control. Six days post challenge all birds were sacrificed. Development of immunity was measured by weight gain (day 0-6 post challenge) and lesion scores.

RESULTS

Table 1 shows the mean oocyst count, the mean weight gain and lesion scores in 7 groups of chicks inoculated orally with 10^5 oocysts exposed to different doses of radiation and 2 (positive and negative) control groups. The chicks inoculated with oocysts exposed to 20, 22, or 25 k..Rad (group 7, 8, 9) didn't discharge oocysts in droppings and showed very few lesions in the duodenum. In groups inoculated with oocysts exposed to 5,10,15 and 18 k.Rad, the oocysts were detected in droppings with varying numbers. The number of oocysts output was in revers relationship with the dose of irradiation. Chicks receiving oocysts that had been exposed to 20, 22, and 25 K.Rad had negligible pathological lesions and a significant higher weight gain ($P<0.05$) than chicks receiving viable non irradiated oocysts (group 2). Chicks receiving oocysts that had been exposed to 5 K.Rad. (group 3) had sever lesions and non significant difference in weight gain with the control positive one (3.3) which received viable nonirradiated oocysts (group 2). Chicks receiving oocysts that had been exposed to 10, 15, and 18 K,.Rad (groups 4, 5 & 6) had less sever lesions and significant higher weight gain than chicks of positive control (group 2). On the other hand,

birds of group 6 revealed significant higher weight gain than birds of groups 4&5. In general the pathological lesions and oocysts output from infected birds with irradiated oocysts had a reverse relationship with the dose of irradiation while the weight gain was increased proportionaly with the dose of irradiation.

Experiment 2

Immunization with irradiated oocysts (table 2) resulted in incomplete protection against challenge. Chicks immunized by oral inoculation with graded doses of nonirradiated as well as irradiated oocysts, showed significant ($P<0.05$) higher body weight gain in comparison with nonimmunized • challenged control. The lesion scores of group 3, 4, 5 were 0.7, 0.5 and 1.1 respectively compared with 3.4 in the control group. The weight gain showed a nonsignificant ($P<0.05$) difference between groups 3, 4 which were immunized with irradiated oocysts at 18 and 22 k.Rad respectively. Chicks of Group 5 that were immunized with viable nonirradiated oocysts recorded a significant lower weight gain 58.25 ± 3.1 gr. than that of group 3,4 (immunized with irradiated oocysts either at 18 or 22 k. Rad. 67.5 ± 2.8 and 60.2 ± 2.99 respectively (table 2).

DISCUSSION

In experiment (1) different doses of irradiation 5,10,15,18,20,22 and 25 K.Rad. were used for attenuation of the oocysts. The oocysts were discharged from chicks of groups inoculated with sporulated oocysts exposed to 20 K.Rad or over doses while the chicks inoculated with sporulated oocysts exposed to doses below 20 K.Rad. discharged oocysts with irrevers relationship between the oocysts output and the dose of irradiation. Klimes et al 1972, Jenkins et al 1991a , Mark et al 1993 reported that, high doses of irradiation affect the excystation of sporulated oocysts and subsequenntial release of sporozoites. Our studies resulted in that the exposure of E.oocysrts to radiation was lowering their pathognicity comparison with the nonirradiated oocysts infection. Irradiation of intestinal Eimeria oocysts to 20 K.Rad or over reduce their pathogenicity and this reflected on the body weight gain and the pathological lesion scores which was nonsignificantly lower than that of non infected control group one. Irradiation of sporulated oocysts at a dose below 20 K.Rad was significantly reduced the body weight gain with a different change in gross pathology. The mean lesion scores of chicks receiving oocysts exposed to 10 K.Rad (group 4) was sever (3) although the pathological lesion had a little effect on

weight gain. This results provides strong evidence that irradiation can attenuate intestinal Eimeria oocysts in agreement with that recorded by Hayat 1976, Jungman & Mielke 1989, Jenkines et al.1991b, Ibrahim, et al. 1996, who cleared that the irradiation of Eimeria oocysts reduce their pathogenicity but each species of Eimeria oocysts show different sensitivity to irradiation dose. The results of the second experiment indicated that the inoculation of chicks with graded doses of irradiated or nonirradiated E.oocysts produce incomplete protection against challenge Judged by weight gains and lesion scores. The attenuation of oocysts by exposure to 18 or 22 k Rad., gave non significant difference in weight gain 67.5 ± 2.8 and 60.2 ± 2.99 gr. respectively. In comparison with chicks of (group 5) primarily inoculated with graded doses of viable nonirradiated oocysts, the weight gain was significantly lower 58.25 ± 3.1 gr.than that of (group.3 &4). This reduction in body weight in group 5 might be due to the high pathogenicity of the primary dose used for immunization which was represented in the lesion score 1.1 compared with 0.7 and 0.5 in chicks of group 3 and 4 respectively. In spite of weight gain in chicks of group 4 (22 K.Rad) was lower than that of group 3 (18 k.Rad) , the dose of 22 k Rad was prefered for immunization than 18 k.Rad because the oocysts were not produced after primary immunization (Jenkines,et al a&b1991).

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(Table 1) showing the mean oocyst count, mean weight gain and avarage lesion scores in the 7 groups of chicks inoculated orally with 10^5 oocysts exposed to different doses of radiation and 2 control groups.

Gr.	Oocyst infection	n dose K.Rad	Mean oocyst output /gr. faeces after 6-14 day P.I (millions)	average (gr.) weight gain	No. Of chicks with a lesion score of									mean lesion score
					0	.5	1	1.5	2	2.5	3	3.5	4	
1	- (negative control)	-	not detected	90 ± 2.999 A	10									0
2	+(positive control)	0.0	4.5	34.5 ± 2.602 D							4		6	3.6
3	+	5	3.5	40.5 ± 2.273 D						1	4	2	3	3.3
4	+	10	2.1	55.6 ± 3.643 C						3	5		2	3
5	+	15	1.1	61.9 ± 2.377 C				1	3	2	4			2.4
6	+	18	1.3	71.8 ± 4.327 B			3	2	2	3				1.7
7	+	20	not detected	83.1 ± 3.531 A	2	1	5	2						0.85
8	+	22	not detected	82.1 ± 2.757 A	3	2	4	1						0.65
9	+	25	not detected	83.8 ± 2.329 A	5	3	1	1						0.4

(table 2) The average weight gain and mean lesion score of chicks immunized with graded doses of oocysts exposed to 0, 18 and 22 K.Rad. and challenged by 10^5 viable sporulated oocysts.

Group No.	Treatment		Average weight gain (gr.)	No. Of chicks with a lesion score of									mean lesion score
	Immunization with	Challenge with 10^5 oocysts		0	.5	1	1.5	2	2.5	3	3.5	4	
1	non immunized	non (negative control)	69.95 ± 3.221 A	10	0	0	0	0	0	0	0	0	0
2	non immunized	+ (positive control)	26.5 ± 2.277 C						2	5	6	7	3.4
3	irradiated oocysts at dose 18 K.Rad.	+	67.0 ± 2.76 AB	5	8	5	2	1					0.7
4	irradiated oocysts at dose 22 K.Rad.	+	60.2 ± 2.994 AB	6	5	4	4	1					0.5
5	Viable oocysts	+	58.25 ± 3.126 B	2	8	3	2	2	1				1.1

A,B,C,D Significant difference limit between groups at 5%