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EFFECT OF CHICKEN ANAEMIA VIRUS (CAV) INFECTION ON CHICKEN COCCIDIOSIS

(With 2 Tables)

By

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أثر العدوى بفيروس أنيميا الدجاج على مرض الكوكسيديا الأعورية فى الدجاج

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حقن الكتاكيت بفيروس أنيميا الدجاج فى عمر يوم عمل على زيادة التأثير الباثولوجى للعدوى بحويصلات الكوكسيديا الأعورية مما أدى الى نقص معنوى فى معدل زيادة الأوزان وشدة التغير الباثولوجى وكذلك زيادة عدد الحويصلات الناتجة فى البراز. كما وجد أن العدوى بالفيروس نتج عنه تأثير مثبط للمناعة المكتسبة من حقن الدجاج بجرعات متتالية لعدد قليل من حويصلات الكوكسيديا الأعورية كان ذلك واضحاً عند إجراء اختبار تحدى المناعة حيث أن معدل الصد كان أقوى فى الكتاكيت الخالية من العدوى بالفيروس عنه فى المجموعات التى حقنت بالفيروس.

SUMMARY

Day old chickens infected with the CAV were exposed to caecal Eimeria oocysts by crop inoculation. Results revealed that CAV aggrivated the pathogenicity of caecal Eimeria infection reflected on the production performance, pathological lesions and the number of discharged oocysts in dropping. Chicken immunised in presence of CAV infection showed low protection rate againest challenge than that resulted from immunization in absence of CAV infection.

Key words: *Chickens - Coccidiosis - Anaemia virus.*

INTRODUCTION

Coccidial infections are self-limiting and depend largely on the number of oocysts ingested, and cell-mediated mechanisms involved in anti-coccidial immunity, which plays a major role in protection (Lillehoj, 1987; Rose, 1987 and Wokelin and Rose, 1990). Marek's disease may interfere with development of immunity to coccidiosis (Biggs *et al.*, 1969), infectious bursal disease may exacerbate coccidiosis (McDougald *et al.*, 1979), and infection with chicken aneamia virus (CAV) Yuasa, (1980). The virus may lead to depletion of cortical thymocytes and a marked depression in T-cell mediated immune response (Engstrom *et al.*, 1988; Otaki *et al.*, 1988; Goryo *et al.*, 1989; Jeurissen *et al.*, 1989; Lucio *et al.*, 1990; Jeurissen *et al.*, 1992, and Hu *et al.*, 1993). The purpose of this study was to define the role of chicken anemia virus infection on chicken coccidiosis and its effect on the immune response of vaccinated chickens against caecal coccidiosis.

MATERIALS and METHODS

One day old balady chicks were obtained from commercial hatcheries. They were raised in wire floored cages,to prevent accidental infection. Commercial ration free from anticoccidial drugs and clean water were provided. All chicks were monitored by sugar flotation of faeces and microscopic examination before experimental coccidial infection.

Eimeria used for immunization and infection:

Caecal Eimeria used in this studies was freshily collected from the caecum of infected broilers. Caecal content was placed in tap-water, mixed and homogenized, centrifuged at 1000 R/M for 10 minuts. The resulting pellet were suspended in saturated magnesium sulphate solution (specific gravity 1.7), and centrifuged. The surface layer containing the oocysts was taken off and diluted with distilled water to reduce its density. After a further centrifugation for 10 minutes. The oocysts pellet was resuspened in distilled water. The centrifugation resuspension cycle was repeated three times to remove residual magnesium sulphat. Washed oocysts were suspended in 2.5% W/V potassium dichromate solution and incubated at 25°C with aerotion for sporulation.

Sporulated oocysts in potassium dichromate solution preserved at 3°C until used..

For preporation of sporulated oocysts for inoculation. The potassium dichromate was removed by three cycles of washing and centrifugation in distilled water. Number of oocysts per ml of suspension was determined by counting in McMaster chamber.

Virus:

Chicken anaemia virus used is GIFU-1 strain was obtained from Yuasa *et al.* (1979). National institute animal health, Japan. The virus was propogated on cell line MDCC-MSB, tumer cell line obtained from Marek's lymphoma according to Goryo et al., (1987).

Hematocrits:

Day old chickens were bled from the wing vein directly into heparinized capillary. The PCV was determined following centrifugation.

Parameters used to evaluate the pathogenicity of Eimeria infection and the immune responce of chickens immunised againest coccidiosis, were Body weight gain. Chickens were weighed indevedually at time of oocysts inoculation and 7 day later and lesion scores. Lesion scores were done on all birds indevedually day after infection using the method deseribed by Johnson and Reid (1970).

Experimental infection of chicks with CAV was verified by anemia and thymic atrophy (Yuasa *et al.*, 1979; Goodwin *et al.*, 1989 and Bounous *et al.*, 1995). For statestical analysis of data. The ANOVA (Duncoan's multiple-range) test at 5% level was used.

Experimental design:

Experiment No. 1 were designed to study the pathogenicity of low and high doses of caecal Eimeria on CAV infected chickens. A total of 180 (day old chicks) were devided into 6 groups (1-6) each contained 30 birds. Chicks of groups 1, 3 and 5 were intra abdominally injected at 1-day-old with 0.2 ml containing 10^6 $TCID_{50}$ of CAV. At two weeks old. Chicks of groups 1 & 2 recieved a single dose of 5×10^3 oocysts, while chicks of groups 3 & 4 were received 5×10^4 viable sporulated oocysts of the locally isolated caecal Eimeria, via direct injection through the skin into the crop using a syring with a 21-gouge needle as described by Johnson and Long (1989). While group 6 was left as negative control.

Ten birds from each group were sacrificed seven days post coccidial infection and the caecum of each bird was examined for pathological scoring using a standard scoring procedure by Johnson and Reid (1970). Weight gain during the seven day infection period was calculated and the thymus weight

of indevedual birds were recorded for each chicken. The other 20 birds from each group were left under observation for other 2 weeks. The dropping of each group were collected daily in 2.5% potassium dichromate and the number of oocysts per gramm were counted for 5 days using the McMaster technique. Haematocrit values were determined indivedually for chicks of all groups 2 weeks post CAV infection. The second experiment were designed to study the effect of CAV infection on the immune responce of chicken immunised against coccidiosis. A total of 150 day old chicks in 5 equal groups were used. At day old, chicks of groups 1 and 3 were inoculated intra-abdomunally with 0.2 ml containing 10^6 TCID of CAV. Chicks of groups 1 & 2 were daily inoculated with 200 viable sporulated oocysts of caecal Eimeria (Local isolates) for 10 days from 6^{th} - 15^{th} day old. Two weeks later, each bird of groups 1-4 were challenged with 1 X 10^5 sporulated oocysts of the same species of Eimeria. Chicks of group 5 left as negative control. Seven days post challenge, 10 chickens from each group were sacrificed for lesion scoring and the thymus weight were recorded for each birds of all groups. Weight gain during the challenge time were indevedually calculated for all chickens. The other birds of each group were left under observation for other 2 weeks. The droppings were collected daily starting from the 6^{th} day post challenge and quantitavely examined for oocysts output for 5 days using McMaster technique.

RESULTS

Results of experiment No. 1 are shown in Table (1) the mean weight gain of chicks given low dose ($5x10^3$) of caecal Eimeria (group 2) in absence of CAV infection were two times (61.6 ± 2.6 gm) greater than those given the same dose of Eimeria in presence of CAV infection group (1) (31.5 ± gm) there was a great difference between this two group in the number of oocysts out put. In group (1) was 9.2 X 10^4/gm Compaird with $5.3x10^4$/gm in group (2) with no gross pathological changes in this two groups. Similar significant higher weight gain in chickens given a single dose of $5x10^4$ oocyst alone (16.7 ± 1.4 gm) group (4) than those in group (3) (7.3 ± 1.2 gm) that infected with the same dose of Eimeria in presence of CAV infection with a minor difference in lesion scores. The number of oocyst out put were $12.1x10^4$/gm in group 4 and $13.4x10^4$/gm in group (3). Mortalities were 15% in group (3) compaired with 5% in group (4).

In the absence of coccidial infection chicks infected with CAV group (5) showed significantly lower weight gain than in control group (6). Two weeks old, chicks of all groups (1, 3 & 5) inoculated with CAV either with or without coccidial infection appeared aneamia with PCV less than 24% Significant atrophy in the thymus gland were observed in chicks of all groups infected with CAV.

In experiment 2 results were ilesterated in Table (2) the chicks immunized with vaiable spourlated oocyst of caecal Eimeria and challenged with 10^5 oocysts of the same species, 2 weeks later in presence of CAV infection (group 1) showed significant high body gain than those in (group 2) that immunized and challenged in absence of CAV infection, with no mortalities in the two groups (1 & 2). There was a minor difference in lesion scores 7 days post challenge and the discharged oocyst were $14.8x10^4$/gm in group 1 compared with $13.9x10^4$/gm in group 2.

Chicks infected with $1x10^5$ sporulated oocyst at 30 day old with CAV infection at 1 day old (group 3) showed weight gain 3.1 ± 2.1 gm compaired with 11.5 ± 1.8 gm in those infected with the same dose of Eimeria only with 20% mortalities in group 3 and 10% in group 4. The number of discharged oocysts in dropping were $15.2x10^4$/gm in group (3) and $11.1x10^4$/gm in group (4). No oocysts were discharged in dropping of control group (6) with no pathological change in the caecum and the weight gain were 69.1 ± 2.9 gm. Chicks inoculated with CAV (groups 1 & 2) showed low PCV (lower than 23%) with great atrophy in the thymus gland.

DISCUSSION

In the present study, we described how an experimental infection with low and high doses of caecal Eimerica in presence of CAV infection leads to more problems than those observed in the control group which recived Eimeria only. The present study in experiment No. (1) proviodes strong evidence that CAV infection aggravate caecal coccidiosis in broiler chickens. Infection of chickens with $5x10^3$ oocysts of caecal Eimeria in absence of CAV infection group (2) dedn't produce significant difference in weight gain and lesion scores than those in non infected control group (6). Infection of chickens with CAV aggrevated the pathogenicity of caecal Eimeria ($5x10^3$ oocyst) group (1) and this reflected on the weight gain that significantly reduced than those in chickens infected with Eimeria only group

(2), while the number of discharged oocysts were higher than in group (1) with a minor difference in the pathological lesions. In chickens inoculated with $5x10^4$ oocysts in presence of CAV infection group (3) the weight gain were reduced and the number of discharged oocysts were high in birds inoculated with $5x10^4$ oocysts in absence of CAV infection group (4) with a minor difference in pathological lesions but mortalities in group (3) reached 15% compaired with 5% in group (4). This finding suggested that the infection of CAV was aggravate the pathogenicity of caecal Eimeria infection. The aggravating effect of CAV on caccidiosis can be due to its immuno suppressing effect of the virus by impairment of T. cell mediated immunfunction Otaki *et al.*, 1988; Adair *et al.*, 1991 and McConnell *et al.*, 1993. With respect to immunosuppression we have noticed marked atrophy of the thymus gland at necropcy and anemia with PCV less than 24% two weeks post CAV infection in chicks of group (1, 3 and 5) that infected with CAV. The aggravating effect of CAV on the pathogensis of other pathogens in the field has been amply demonstrated (Engstrom and Luthman, 1984; Bulow *et al.*, 1986; Yuasa *et al.*, 1987; Engstrom *et al.*, 1988 and McNeilly *et al.*, 1995).

Our results of the experiment No. 2 are shown in Table (2) indicat that significant atrophy in the thymus gland of chicks infected with CAV (groups 1 and 3) and the hematocrit value of this chicks were less than 23% this chicks regarded as anemmic (Yuasa *et al.*, 1979). This effect of CVA on the thymus gland may be the underlaylig caus of impaired the cell-mediated mechanism that plays a major role in anticoccidial immunity. This immunosuppression reflected on the protection of immunized chickens againest challenge. Chickens immunized in presence of CAV infection (group 1) showed low protection rate againest challenge than that resulted from immunization in absence of CAV infection (group 2) and vice versa in the number of oocyst out put post challenge our results are in agreement with that reported by other workers, who showed that the infection of chickens with CAV was impaired respone to vaccination of Marek's disease (Bulow *et al.*, 1983) and inactivated Newcastle vaccine (Box *et al.*, 1988).

In conclusion, results of experiments make likely that the infection of chicken with CAV aggravating caecal coccidiosis and play role in vaccination failier by its effect on the thymus gland.

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Table (1): Shows some obseved paameters of chicken inoculated with CAV at day old and injected with different doses of caecal Eimeria at 15th of age.

Group No.	Treatment		Mean weight gain during 7 days infection	No of birds with a lesion score of						No of oocyst output x 10^4/gm feaces	PCV	Thymus body weight ratio	Morta-lities
	CAV infection at day old	No of oocyst inoculated		0	1	2	3	4	Mean				
1	+	5×10^3	31.5 ± 3.4^{C}	4	5	1			0.7	6.2	23.8 ± 0.9^{BC}	0.20 ± 0.02^{CD}	0
2	-	5×10^3	61.7 ± 2.6^{AB}	5	4	1			0.6	5.3	30.0 ± 0.6^{A}	0.58 ± 0.02^{A}	0
3	+	5×10^4	7.3 ± 1.5^{E}				2	8	3.8	13.4	20.7 ± 0.9^{D}	0.18 ± 0.01^{D}	3/20
4	-	5×10^4	16.2 ± 1.4^{DE}				5	5	3.5	12.1	30.8 ± 1.3^{A}	0.61 ± 0.02^{A}	1/20
5	+	-	51.8 ± 1.7^{B}	10					0	0	22.3 ± 0.9^{CD}	0.22 ± 0.02^{BCD}	0
6	-	-	65.3 ± 2.7^{A}	10					0	0	29.7 ± 0.7^{A}	0.60 ± 0.01^{A}	

A-E Significant difference limit between groups at 5%

Table (2): Shows some observed parameters of chickens inoculated with CAV at day old, immunized with 10 repeated dose of 200 oocysts and challenged 10^5 oocysts of cecal Eimeria.

Group No.	Treatment			Mean weight gain during 7 days challenge	No of birds with a lesion score of						No of oocyst in droping $x10^4$/gm	PCV	Thymus body weight ration	Morta-lities
	CAV infection at day old	Immuni-zation	Challenge		0	1	2	3	4	Mean				
1	+	+	+	28.1 ± 3.4^{B}		5	5			1.5	14.8	22.5 ± 0.8^{BC}	0.15 ± 0.07^{D}	0
2	-	+	+	66.8 ± 2.2^{A}		6	4			1.4	13.9	30.0 ± 1.4^{A}	0.59 ± 0.01^{B}	0
3	+	-	+	3.1 ± 2.1^{D}				4	6	3.6	15.2	21.2 ± 0.1^{C}	0.19 ± 0.01^{CD}	5/20
4	-	-	+	11.5 ± 1.8^{C}				6	4	3.4	12.1	30.3 ± 0.9^{A}	0.65 ± 0.02^{A}	2/20
5	-	-	+	69.7 ± 2.9^{A}	10					0	0	30.0 ± 0.9	0.64 ± 0.08^{AB}	0

A-D Significant difference limit between groups at 5%.