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EFFECT OF TOLTRAZURIL AND AMPROLIUM PLUS IN BROILER CHICKENS INFECTED WITH COCCIDIOSIS

(With 5 Tables)

By

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تأثير التولترازوريل والأمبرول بلس فى بدارى دجاج التسمين المصابة بالكوكسديا

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أجريت هذه الدراسة لتقييم الاستعمال المتزامن للتولترازوريل والأمبرول بلس سوياً فى علاج الكوكسيديا فى هذه الدراسة استعمل ٢٠٠ كتكوت هبرد عمر يوم تم تقسيمهم إلى خمس مجموعات متساوية (الأولى ضابط سلبى للتجربة والثانية ضابط إيجابى، والثالثة والرابعة والخامسة جرعت فى عمر ١٩ يوم (10^4) حويصلة متجرثمة من خليط من الأيميريا٠ تم معالجة المجموعة الثالثة للتولترازوريل ٢٥ مجم / لتر ماء شرب وتم معالجة المجموعة الرابعة بالأمبرول بلس بمعدل ٢٥٠ جزء فى المليون على العلف والمجموعة الأخيرة تم معالجتها بالتولترازوريل والأمبرول بلس سوياً بالجرعات السابقة٠ تم تقييم القدرة العلاجية للأدوية المستعملة من خلال تقييم المعايير الآتية (عد حويصيلات الأيمريا فى زرق الدجاج المعالج بعد ٢، ٦، ١١، ١٦ ، ٢١ يوم من العدوى وأثناء العلاج، كذلك تقييم معدلات الأداء الإنتاجى المختلفة فى عدة مراحل من التجربة وفى عمر ٤٠ يوم تم وزن ثم ذبح خمس طيور من كل مجموعة وتم تجميع عينات الدم منهم التى قسمت بدورها إلى جزئين جزء أخذ على مانع للتجلط لدراسة صورة الدم وجزء آخر تم فصل المصل منه لدراسة وظائف الكبد والكلى وعمل أختبار منع التلازن الدموى ثم وزن الكيس الفبريشي لتحديد معامل وزنه إلى الجسم كأحد دلالات التنبيط المناعى٠ وفى عمر ٤٠ يوم تم تعريض ١٠ طيور من الطيور المتبقية فى كل مجموعة إلى عدوى النيوكاسل الحشوى الضارى بجرعة 10^6 / طائر حقناً فى العضل لتقييم التنبيط المناعى أيضا٠ من النتائج التى تم التوصل إليها

يمكن القول أن أستخدام التولترازوريل والأمبرول بلس بالجرعات العلاجية كان له أثراً جيداً في تحسين الحالة الصحية للطيور من حيث خفض الوفيات وتحسين معدلات الأداء الإنتاجي دونما أن يؤثرا سلبياً على وظائف الكبد والكلى أو ان يكون لهم تأثيراً مثبطاً للمناعة وأن هذا التأثير كان جلياً عند الاستخدام المتزامن لكلا العقارين.

SUMMARY

The present study was performed to evaluate the concurrent use of toltrazuril and amprolium plus in treating avian coccidiosis. Two hundred one day old Hubbard broiler chicks kept in wire floor batteries under hygienic measures were used. On day 19th of age chicks were aliquated into five groups, 40 each (1st group served as negative control, 2nd group served as positive control, 3rd group was infected and treated with toltrazuril 25 mg/L of drinking water, 4th group was infected and treated with Amprolium plus 250 ppm in ration and the 5th group was infected and concurrently treated with toltrazuril 25 mg/L and amprolium plus 250 ppm in ration. Treatment usually started 3 days post infection, chicken in all groups were weighted at 20, 27, 34 and 41 day of age. Body weight gain, feed consumption, feed conversion, mortality, lesion score was determined. Oocyst count was performed 2, 6, 11, 16 and 21 days PI. On the 40th day of age, 5 birds from each group were weighted, slaughtered and had bursal body weight index determined. Blood samples from slaughtered birds were divided into two portions, one for serum preparations for serology and clinical chemistry parameters, while the other portion was collected on EDTA for hematological study. 10 of the remaining birds from each group were subjected for virulent Newcastle diseases challenge. Results obtained showed that tolrazuril and amprolium plus administration individually at the therapeutic doses had valuable effect in the treatment of avian coccidiosis and simultaneous administration of both drugs had more potent curative effect and less effect on liver and kidney function parameters, meanwhile non of the used drugs proved immunosuppressive.

Key words: *Coccidiosis, broiler chickens, toltrazuril, amprolium plus*

INTRODUCTION

Coccidiosis is a disease of universal importance in poultry production, it causes intestinal damage with resulting interruption of feeding, digestive processes, nutrient absorption and dehydration, beside blood loss and increased susceptibility to other diseases agents (Mc Dougald 2003).

Eimeria spp. causing coccidiosis in chickens are ubiquatous, and it has been said that, the only limit to their distribution is the distribution of their host (Jordan and Pattison 1996).

Several key factors are responsible for the epidemiology of coccidiosis and its ubiquitous nature. These are, the long persistence of Oocyst in the environment, the short prepatent period and the high biotic potential of the parasite. There is no maternally derived immunity for early protection and that immunity is acquired by infection and maintained by continual infection., and the attak with one spp. of parasite does not confer full protection against another spp. Coccidial oocyst can tolerate most disinfectants and only ammonia and methylbromide gas can effectively kill oocysts but they are highly noxious for poultry and working personnel thus they can not be used during disease outbreak. The moisture, oxygen and warmth required for sporulation are similar to those required for sound poultry breeding (Jordan, 1990; Jordan and Pattioon 1996; Alexander, 2002; McDougald 2003).

Control of coccidiosis relies principally on drugs Alexander (2002), Amprolium and toltrazuril are the usual drugs used during outbreak of coccidiosis, but development of tolerance for drugs after exposure in a serious limitation for the effectiveness of drug (McDougald, 2003).

This work, was planned to evaluate the simultaneous use of toltrazuril (Tol) and amprolium (Amp) plus in treating avian coccidiosis through the parasitological clinicopathological studies as well as changes in body weight, oocyst output, lesion score, mortality rate and possible immunosuppression in broiler chickens.

MATERIALS and METHODS

Materials

Chickens:

Two hundred day old Hubbard broiler chicks, obtained from Cairo poultry company were used in the present study.

Drugs:

Toltrazuril

Toltrazuril (Bayer) 2.5% was used at a dose of 25 mg / L of drinking water (Vertommen and Peek 1990).

Amprolium plus:

Amprolium plus (merial) each gm contains 250 mg amporlium HCL and 16 mg ethopabate , the drug was used at a dose of 250 ppm.

Ration:

High energy, high protein ration "super starter ration" Cairo poultry company was used ad libitum.

Mixed Eimeria sporulated oocysts:

Intestinal contents from field cases suffering coccidiosis submitted to the animal health res. Lab. Zagazig was preserved in 4% potassium dichromate and aerated with aquarium pump to promote sporulation as described by McDougald (2003).

Viruses:

Live virus vaccines:

NDV (HB_1, and LaSota) intervet, IBDV (D-78) intervet were used according to their manufactures.

Challenge NDV:

A VVNDV with a titer of $10^{7.7}$ EID_{50} /0.1 ml Bayoumie *et al.* (2006) was used.

Chicken embryos:

Nine day old chicken embryos from native balady breeding flock reared in Sharkia was used for virus propagation and antigen preparation.

Viral antigens:

ND Hemagglutination antigen:

Allantoic fluid from chicken embryos inoculated with Losata NDV was used as hemagglutinating antigen for HI – test (Allan *et al.* 1978).

ND precipitating antigen

The CAM of chicken embryos inoculated with Lasota NDV was prepared as described by Bayoumie (1997) and used as ND precipitating antigen.

ND precipitating antiserum:

Serum was prepared earlier (Bayoumie *et al.*, 2006).

Methods:

Experimental design:

Table 1 summarizes the distribution of experimental birds and experimental design.

Table 1: Outlines the performed experimental procedure

Group	Number	Experimental groups	Preventive vaccination		Inoculation schedule	Sampling & experimental procedure	
			Age	Vaccine		Calender	Procedure
A	40	Negative control- non infected no treated (but vaccinated)	7D	HB, eye drops	Group B,C,D,E Were crop inoculated with 10^4 sporulated Eimeria oocyst /bird	At 20,27,34 & 41 days of age	B.W., WG, FCR
B	40	Positive control infected non treated	14D	IBDV eye drops		2,6,11,16 & 21 day PI	Oocyst output count
C	40	Infected, treated with toltrazuril 25 mg/L drinking water	18 D	Lasota D.W.		At 40 day of age	-Blood collection on EDTA for hematology -Serum collection for AST, ALT, ALP, GGT uric acid creatinine & HI -B.B. index -VVNDV challenge 10^6 VP/IM
D	40	Infected , treated with amprolium 250ppm/ kg feed					
E	40	Infected, treated with (Tol+ Amp) simultaneously					

BW= Body weight, WG= weight gain, FCR=feed conversion ratio, VP= virus particle, IM= Intramuscular , AST=Asptrate immunotrasnferase , ALT=alanine aminotransferase, ALP= Alkaline phosphatase , GGT= gama glutamy transferase , HI= haemaggultination inhibition , BB index= bursal body weight index , VVNDV= velogenic viscerotropic Newcastle viruses

Experimental coccidial infection:

On 19th day of age group B, C, D, E were crop inoculated with 1 x 10^4 mixed eimeria oocysts. (Karim and Tress, 1990).

Coccidal lesion score:

It was performed according to Johnson and Reid (1970).

Productive performance parameters:

Body weight (BW), weight gain (WG) and feed conversion rate (FCR) were performed for all chicken groups at 20, 27, 34 and 41 day of age.

Oocyst output count:

At 2, 6, 11, 16 and 21 day PI oocyst output was counted using McMaster Technique as described Johnson and Reid (1970).

Hematological and clinicochemical parameters:

At 40 day of age blood was collected on EDTA for determination of hemogram and total leukocytic count according to Coles (1986), another part of the blood sample was used to obtain serum for determination of serum transaminases (AST – ALT) (Reitman and Frankel 1957), Gamma glutamyltransferase (GGT) (Szaz, 1969), serum alkaline phosphatase Tietz (1986), serum uric acid (Caraway, 1957), creatinine (Husdan and Rapoport 1968).

Hemagglutination and hemagglutination inhibition:

They were performed as described by Villegas (1991).

Evaluation of immunosuppression:

Immunsuppression due to IBDV vaccination and treatment with Toltrazuril and amprolium was tested by serologic and challenge results, beside bursal body weight index (Lucio & Hitchner 1979).

Statistical analysis:

Statistical analysis was done after Snedecor and Cochran (1967).

RESULTS

Results of the present work are illustrated in Tables (2-5)

Table 2: Shows mortality, lesion score and oocyst count in the different experimental groups 2, 6, 11, 16 and 21 day post infection.

Experimental groups	Total number	Mortality		Lesion score	Oocyst count in gm faeces x 10^3				
		Number	%		2 D.PI	6D.PI	11D.PI	16D.PI	21D.PI
a. Non infected non treated	40	0	0	0	0	0	0	0	0
b. Infected non treated	40	4	10	4	2.22± 0.08	4.17± 0.18	5.49± 1.15	7.27± 0.73	7.52± 1.36
c. Infected treated with (Tol)	40	0	0	2	0.52± 0.09***	0.36± 0.16***	1.74± 0.3***	2.71± 0.78***	2.07± 0.59***
d. Infected treated with (Amp)	40	1	2.5	2	0.52± 0.11***	0.33± 0.32***	1.82± 0.3***	2.05± 0.3***	2.26± 0.18***
e. Infected treated with (Tol & Amp)	40	0	0	1	0.61± 0.3***	0.24± 0.19***	1.44± 0.2***	1.99± 0.16***	1.31± 0.17***

***= High significant P<0.001

Table 3: The hematological findings, liver and kidney functions in the different experimental groups PI at 40 days of age.

Group	Hematological findings							Liver functions				Kidney function	
	RBCs x 10^6/ml	Hb%	PCV	WBCs x 10^3/ml	MCV	MCH	MCHC	ALT µ/ml	AST µ/ml	ALP	GGT	Uric acid mg/dl	Creatinine Mg/dl
a	3.04± 0.2	8.97± 0.74	30.04± 1.21	31.82± 0.92	98.8	29.5	29.8	28.19± 1.23	30.76± 1.87	23.16± 1.31	21.08± 1.73	8.03± 0.94	1.02± 0.08
b	2.1± 0.34	7.06± 0.17	27.17± 0.22	34.27± 0.26	129.3	33.61	25.0	35.32± 0.83**	39.28± 0.94**	29.34± 0.87**	25.26± 0.26**	10.73± 0.36**	1.48± 0.1*
c	2.92± 0.48	8.03± 0.32	28.62± 0.28	32.18± 0.61	98	27.5	28	31.21± 0.31	34.18± 1.38	25.92± 0.38	23.61± 0.21	9.73± 0.14	1.23± 0.12
d	2.91± 0.34	8.18± 0.27	28.77± 0.62	33.06± 0.65	98.8	28.1	28.4	32.36± 0.88	35.21± 0.58	25.61± 0.13	23.83± 0.18	9.73± 0.14	1.26± 0.09
e	2.96± 0.42	8.62± 0.89	28.98± 0.73	31.97± 0.72	97.9	29.1	29	30.94± 0.93	31.91± 0.18	22.7± 0.25	22.14± 0.21	9.73± 0.14	1.13± 0.21

* Low significant P= <0.05 ** Moderate significant P<0.01

Table 4: Average body weight (ABW), weight gain (WG), feed consumption (FC), feed conversion rate (FCR) in the different experimental groups.

Parameters / Groups	A.B.W. at 20 days	27 days				34 days				41 days			
		A.B.W.	W.G	F.C.	F.C.R	A.B.W.	W.G	F.C.	F.C.R.	A.B.W.	W.G	F.C.	F.C.R.
Non infected non treated	574.21 ± 3.21	874.28 ± 5.28	300.07 ± 4.29	680.21	2.27	1323.26 ± 5.27	448.98 ± 2.51	900.24	2.005	1805.18 ± 4.38	481.92 ± 2.53	940.46	195
Infected non treated	573.24 ± 4.32	853.15 ± 6.25*	279.91 ± 4.23*	664.35	2.38	1295.96 ± 3.34**	442.81 ± 2.27*	899.48	2.03	1766.04 ± 6.36**	470.08 ± 2.09*	928.57	1.98
Infected treated with (tol)	579.51 ± 4.42	894.38 ± 3.27**	314.87 ± 3.29*	684.07	2.17	1346.18 ± 5.37*	451.8 ± 4.31	912.09	2.01	1833.27 ± 5.27	487.0 ± 2.45*	942.48	1.94
Infected treated with (amp)	576.36 ± 3.72	890.92 ± 6.37*	314.56 ± 3.48*	682.19	2.17	345.46 ± 4.39*	454.54 ± 4.29	920.39	2.02	1831.38 ± 5.51	485.29 ± 2.09*	942.93	1.94
Infected treated with (tol ± amp)	579.43 ± 4.398	897.27 ± 4.19**	317.87 ± 4.71*	687.59	2.16	1355.26 ± 3.57**	457.99 ± 3.57	922.52	2.01	1846.59 ± 4.43	491.33 ± 1.64	941.38	1.92

* Low significant P= <0.05 ** Moderate significant P<0.01

Table 5: Shows mean HI, results of challenge with VVNDV, bursal weight, body weight, bursal body weight ratio and bursal body weight index in the different experimental groups at 40 day of age.

Group	Mean HI titer	Protection % post VVNDV challenge	Mean bursal weight	Mean body weight	Bursal body weight ratio	Bural body weight index
a	2.947±0.4	90%	1.9±0.5	1805±4.38	0.00105	
b	2.5±0.3	70%	2.1±0.2	1766±6.36**	0.0011	1.132
c	2.9±0.29	90%	1.9±0.4	1833±5.27	0.00103	0.95
d	3.1±0.4	90%	2.1±0.3	1831±5.51	0.00114	1.08
e	3.0±0.38 n.s.	90%	2.3±0.2 n.s.	1846±4.43	0.00124 n.s	1.186

** P<0.01

DISCUSSION

Weight gain and coccidial lesion score are primary criteria for measuring the anticoccidial efficacy Conway *et al.* (1990) .In the present study clinical signs, mortality rate, lesion score, oocyst count, performance and hematological picture was performed, beside the liver and kidney functions and some immunological criteria, to examine the safe usage of the tested drugs.

Clinically the positive control group (b) showed reduced feed intake, depression, ruffled feather, debility, dropped head, blood diarrhea, 10% mortality, the highest lesion score and the highest oocyst count post infection (PI). (Table 2). These findings are in agreement with the previous findings of Ahmed (2004) and Eid *et al.* (2004).

The hemogram of negative control group (a) RBCs count, HB%, PCV and WBCs count were similar to those reported by Twisselmann (1939); Pilaski (1972); Lucas and Jamaroz (1961) and Olson (1937) respectively. RBCs count, HB%, PCV values were significantly lower in group (b) (Table 3). this is due to hemorrhagic enteritis due to coccidial infection (Conwary *et al.*, 1993; McDogald, 2003; Eid *et al.*, 2004; Seddik and El-Bealawy 2007), while the significant increase in the total leucocytic count may be due to enteritis specially if we consider that this group was showing the highest enteric lesion score (Table 2). Similarly found Seddik and El-Bealawy (2007).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are transaminases enzymes, they had great clinical importance in the inter conversion of aminoacid and oxoacid by transfer of amino group. ALT increases due to pathologic changes in all tissues. AST activity greater than 23 u/L are considered abnormal and it reflects repatic, skeletal muscles or kidney changes. Alkaline phosphatase (ALP) in involved in energy transfer for exchange of ions across the cell membrane, this activity is present in liver, intestine and bone. Elevation of its level indicates cellular damage (Lumeij, 1988; El-Sayed 2002). Gamma glutamy transferase (GGT) cleaves the Gamma glutamyl group from peptides and moves them to an appropriate acceptor, these enzymes are of great importance in biliary and renal tubular epithelium. (GGT) activity in the serum is from biliary origin (Hochleithner, 1994). (AST), (ALT), (ALP) and (GGT) could be used as indicative for hepatic pathology (Lewandowoki *et al.*, 1986).

In the present study negative control group (a) showed the lowest value for (AST), (ALT) (ALP) and (GGT) (Table 3). Positive control group (b) showed moderate significant increase in AST, ALT, ALP and GGT (Table 3). Similar to the previous findings of Fukata *et al.*, (1997); Eid *et al.* (2004) and Sameh *et al.* (2005) this could be attributed to the dehydration resulting from diarrhea caused by coccidia Mc Dougold (2003) group (c,d) receiving toltazuril or amprolium plus individually showed high non significant ALT, AST, ALP and GGT compared to negative control meanwhile group (e) receiving toltrazuril and amprolium plus simultaneously had a non significant low ALT, AST, ALP and GGT as compared with the negative control group or the individually treated groups pointing to the value of concurrent use of the two drugs (Table 3).

Uric acid is the primary catalatic product of protein and non protein nitrogenous in birds, it is excreted by the avian kidney primarily by tubular excretion, therefore elevated serum uric acid values are expected in birds with impaired renal function and reduced renal uric acid clearance. The normal blood uric acid values for most birds ranges between 2-10 mg/dl.

Creatinine is not a major non protein nitrogen component in avian blood, it has a questionable value in evaluating renal function in birds, its normal value in most birds is 0.2 mg/dl. Creatinine well be elevated slightly in renal failure 0.5-1.5 mg/dl but is less reliable than

uric acid in evaluating renal functions, because birds excretes creatine in their urine before it has been converted to creatinine. The measurement obtained as normal for creatinine may be pseudocreatinine such as glucose, protein, ascorbic acid and pyrivic acid thus it may not reflect glomerular function.

In the present study groups (a, b, c, d and e) had serum uric acid values ranging from 8.03 up to 10.73 within the high normal physiologic limits (Table 3) and the creatinine values were ranging from 1.02 up to 1.48 mg/dl, the high values in the non treated group (a) lead us to consider the high protein level of the consumed diet (high energy - high protein), to be the cause for this high measurement of kidney function parameter (Lewandowski *et al.*, 1986).

Treatment of infected chickens with the therapeutic dose of toltrazuril or amprolium plus reduced the clinical signs and improved the health status of infected groups as evidenced by the decreased mortality rate, lesion score and oocyst output. Similar results for amprolium were obtained by Tocchini and Tassi (1984); Chapman (1989); Chapman (1999) and Hernandez *et al.* (2000) while similar results for toltrazuril treatment was obtained by Khaled (1994); Ramadan *et al.* (1997) and Sameh *et al.* (2005).

The reduction in oocyst output following treatment with amprolium is due to its effect on the first generation schizont Danforth and Anderson (1989); Vertommen and Peak (1990) stated that the efficacy of toltrazuril is due to its effect on sexual stages of coccidia and also through the inhibition of nuclear division of schizont.

Performance parameters such as body weight, weight gain, feed consumption, feed conversion rate in experimental groups treated with toltrazuril or amprolium plus were improved when compared to the non infected non treated group. Similarly found Sameh *et al.* (2005) and Mohamed (2008) the improvement of performance parameters may be due to improvement of general health conditions, increase food intake and absorption of nutrients as mentioned by Abdein and Abd El-Fattah (2003), but Joyner *et al.* (1963) stated that this effect would be due to improvement of the integrity of the epithelial lining of the gastro intestinal tract.

In the present study B:B index, challenge results, and serology were used to evaluate immunosuppression after IBDV vaccination or toltrazuril and amprolium medication. B:B index for the different

experimental groups were ranging from (0.95-1.186). Lucio and Hitchner (1979) found that B:B index lesser than (0.7) signifies immunosuppresion this was not encountered in any of the experimental groups.

The protection % against VVNDV was 70% in group (B) this may be due to disease concurrency post coccidial infection as mentioned by Mc Doughald and Reid (1991), in the remaining groups the protection % was (90%). This is correlated with the HI immune titer as mentioned by Allan *et al.* (1978).

From the above mentioned data we could conclude that the tested drugs have no effect on the immune competance this is evident from the results of B:B index, serology, and challenge in the different tested groups.

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