Sensitivity Of Two Local Field Isolates Of *Eimeria Tenella* To Maduramycin And Diclazuril

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ABSTRACT

Two battery trials each comprising 6 treatments with 3 replicates of 10 birds / replicate were carried out to evaluate the efficacy of two anticoccidial drugs (maduramycin and diclazuril) against experimental infection with two local field isolates of *E. tenella* isolated from two different governorates *(Matrouh* and *El-Behera)*. Maduramycin and diclazuril were given in the feed of the birds at concentration of 5 ppm and 1 ppm respectively starting from two days before the infection to two weeks postinfection. Infection was performed with $25x10^3$ sporulated oocysts/bird/os for each isolate at two weeks of age. The performance of experimental birds was observed over 14 days post infection (P.I). According to the parameter used for judgement (Global resistance Index) (GI), the efficacy of maduramycin was between limited (77.83% of NNC) and good (85.05 % of NNC) for *Matrouh* and *El-Behera* isolates, respectively. While diclazuril had good efficacy (81.51 % of NNC) in the control of infection with *Matrouh* isolate and had very good efficacy (92.54 % of NNC) on *El-Behera* isolate.

INTRODUCTION

Coccidiosis remains one of the most expensive and common disease of poultry inspite of advances in chemotherapy, management, nutrition, and genetics ***(1)***.

The wrost effects of cocccidial infection in poultry are reduction of weight gain and an adverse effect on feed conversion ratio ***(2)*** and increased mortalities of 6-10% in broilers ***(3)***. The annual economic losses for poultry production due to coccidiosis are more than US $ 3 billion ***(4)***.

Coccidiosis prevention in broilers is primarily based on inclusion of anticoccidials in the feed ***(5)*** and to a certain extent live vaccines. The current expense for preventive medication exceeds $ 90 million in the United States and more than $ 300 million world wide ***(1)***.

However drug–resistance in coccidial populations has been a constant threat to the continued success of prophylactic chemotherapy ***(6)***. *Eimeria* spp. acquired resistance rapidly against almost all the chemical compounds that have been introduced ***(7,8)***. While, it develps slowly against ionophores ***(9,10)***. Since polyether ionophores constitute more than 80 %, they have been used for more than 30 years but development of resistance to them has become an increasing problem allover the world ***(11-13)***.

Although wide spread use of switching and rotating of anticoccidials during a grow out or between poultry grow out cycles hopes reducing drug resistance, the erosion of coccidial drug sensitivity has continued ***(14)***. Most studies indicated that resistance is stable even in the absence of drug selection pressure ***(15-17)***.

Coccidiosis vaccines technology isn't always effective and does nothing to control necrotic enteritis ***(18)***. Furthermore, the performance of vaccinated broilers was less than non-vaccinated (medicated birds) considering final live body weight and feed conversion ratio ***(19)***. Beside, antigenic variability between the *Eimeria* spp. present in the vaccine and those in the field restricts the effectiveness of commercial vaccines ***(19)***.

So continuous anticoccidial drugs testing is important for selection of effective prevention and control drugs. The present study was designed to determine the sensitivity of two local field isolates of *E. tenella* from two

different governorates *(Matrouh* and *El-Behera)* to nowdays available two commercial anticoccidial agents maduramycin and diclazuril.

MATERIALS AND METHODS

Parasites

Two strains of *E.tenella* were isolated from clinical coccidiosis field cases, strain 1 (*Matrouh*) and strain 2 (*El-behera*) were isolated from two different governorates (*Matrouh* and *El-Behera*) and were propagated from a single sporulated oocyst.

The two isolates were propagated in chickens once before their use as an inoculum ***(20).***

Chickens and Experimental design

Two experiments (Exp.1 and 2) were conducted to assess the sensitivity of two isolates of *E. tenella* to maduramycin and diclazuril, respectively.

Maduramycin (Ionophorous antcoccidial) was tested in experiment 1 and Diclazuril (synthetic anticoccidial) was tested in experiment 2.

In each experiment, a total of 180, one day-old unsexed broiler (Avian 48) chicks were housed in batteries with continuous illumination and kept free from coccidian infection.

At the age of 12 days (D-2), the birds were individually weighed, wing tagged and assigned into 6 treatments with 3 replicates of 10 birds each by ranking method that approximately equalized initial weights after culling of the two weight extremes and distributed the replicates into battery cages randomly ***(20).***

At 14 days of age (D0), all experimental chicks, except the non-infected controls, were infected with $25x10^3$ sporulated oocysts of *E. tenella* / os (two treatments with *Matrouh* isolate and two treatments with *El-Behera* isolate) which were prepared in 1 ml of distilled water using Insulin syringe (without the needle) into the crop directly.The chicks were tested for 3 successive days by flotation method to prove being coccidia-free before infection.

The treatments were as follow:

T1: Non infected, non medicated control (NNC)

T2 : Non infected, medicated

T3: Infected (*Matrouh*), Non medicated control (INC)

T4 : Infected *(Matrouh*), medicated

T5: Infected (*El-Behera*), Non medicated control (INC)

T6 : Infected (*El-Behera*), medicated

Anticoccidial Drugs

The anticoccidials (maduramycin in experiment 1 and diclazuril in experiment 2) were blended into the basal diet at the recommended levels to give final concentrations of 5 ppm maduramycin and 1 ppm diclazuril and given to birds (medicated groups) from D-2 to the end of the experiments (D+14 Post infection)(P.I.).

Feed

The basal diet consisted of anticoccidial free commercial-type starter diet (from Zoocontrol for Industralization and trade). Feed and water were available *ad libitum.*

Parameters

Several criteria were used for evaluation of the efficacy of each drug, these included:

- Body weight, weight gain, feed consumption and feed conversion ratio were performed for all chicks at D0, D+7 and D+14 P.I.
- The coccidial-induced mortality.
- Lesion scoring at 6^{th} day P.I ***(21)***
- Daily oocyst output counts from D+5 to D+14 (P.I) using McMaster technique ***(22)*** and analysed statistically after natural log transformation ***(23).***
- Oocyst index: a semiquantitative scoring system was used ***(24).***
- Faecal score from D+5 to D+10 P.I. was calculated ***(25).***

Furthermore, all criteria were combined to give a single criteria of efficacy Global resistance Index (GI) *(26)* using parameters of Weight gain %, Feed conversion (g / g), lesion scores *(21)*, a semiquantitative oocyst index *(24)*, and mortality % using the following formula:

$GI = \% WG_{NNC} - [(F_G - F_{NNC}) \times 10] - (OI_G - OI_{INC}) - [(PI_G - PI_{INC}) \times 2] - (\%\ mortality / 2)$.

Where GI = the global index, OI = Oocyst Index, WG = weight gain, PI = gross-pathological index, F = feed conversion, G = treatment group, NNC = non-infected/non-medicated control and INC = infected/non-medicated control.

The global index of each tested group was given as a percentage of the global index for the NNC.

The following five categories were used to assess the efficacy of the tested anticoccidials:

Very good efficacy	$\geq 90\% GI_{NNC}$
Good efficacy	$\geq 80\% GI_{NNC}$
Limited efficacy	$\geq 70\% GI_{NNC}$
Partially resistant	$\geq 50\% GI_{NNC}$
Resistant	$< 50\% GI_{NNC}$

Statistical analysis

Data were analyzed using the GLM procedure of SAS® *(27)* . Student Newman Kelus Test *(27)* was utilized to test mean differences at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of wieght gain, feed consumption, feed conversion ratio, daily oocyst count (Log_{10}), mortality rates, lesion scores, oocyst index, faecal scores were all calculated and presented in Tables (1-3) for experiment 1 and Tables (4-6) for experiment 2.

Exp.1:Evaluation of the efficacy of maduramycin

Table 1, revealed that infection with *Matrouh* or *El-Behera* isolates reduced weight gain (g/bird/day) of infected non medicated group INC (T3) or (T5) significantly than non infected, non medicated group NNC (T1) at D0 to D+14 P.I which were 43.7 and 50.27 for T3 and T5, respectively versus 57.4 for T1. However maduramycin improved significantly weight gain of infected birds with (*Matrouh* isolate) (T4) than that of INC (T3). While, there was no improvement in weight gain of infected group with (*El-Behera* isolate) receiving maduramycin (T6) over INC (T5).

Infection with *Matrouh* isolate resulted in decreased feed consumption at D0 to D+14 P.I either with or without anticoccidial drug (T4) or (T3), respectively.

While feed consumption in chickens infected with *El-Behera* isolate, either medicated (T6) and non medicated (T5) groups didn't change significantly when compared to NNC (T1) .

Feed conversion ratio differed significantly between all tested groups at D0 to D+14 P.I except between medicated groups, infected with *Matrouh* and that infected with *El-Behera* isolate (T4) and (T6), respectivly. Which were:1.42, 1.45, 1.73, 1.62, 1.6 and 1.62 among groups 1, 2, 3, 4, 5 and 6, respectively.

It was clear that the improvments in feed conversion in medicated groups, infected with *Matrouh* isolate were consistent with the improvments in weight gain and control of lesion scores and reduction of oocyst shedding.

The results indicated that maduramycin didn't result in any improvments in feed conversion ratio and weight gain of infected groups with *El-Behera* isolate, and this may be attributed to the light infection with *El-Behera* isolate so it didn't affect weight gain and feed consumption significantly when compared to NNC and/or due to the reduced sensitivity of *El-Behera* isolate to maduramycin. These findings were supported by previous studies *(28,29)* which reported that weight gain of challenged and medicated group with maduramycin were as lower as challenged non medicated groups. The same was in our study shown with *El-Behera* isolate.

Depression of weight gain and higher FCR of infected birds with *El-Behera* isolate in the presence of maduramycin than in infected, unmedicated birds suggesting a tendency

toward ionophore dependence as previously reported in *E. tenella* with ionophores *(30)* and in *E. maxima* with robenidine dependence *(31).*

Table 1. Body gain, feed consumption and feed conversion ratio.

Treatment Number	Infection	Medication (maduram-ycin)	Body gain g / bird / day at		Average total gain g / bird / day at D0 to D+14	Feed consumption g / bird / day at		Average total feed consumption g / bird / day at D0 to D+14	FCR* / bird / day at		Average FCR / bird / day at D0 to D+14
			D0 to D+7	D+7 to D+14		D0 to D+7	D+7 to D+14		D0 to D+7	D+7 to D+14	
T1	Non	Non	45.3^{b} ±4.98	75.2^{a} ±2.46	57.4^{a} ±1.87	65.99^{b} ±13.81	110.67 ±3.06	81.53^{b} ±2.80	1.46^{d} ±0.01	1.5^{f} ±0.08	1.42^{e} ±0.002
T2	Non	Yes	54.17^{a} ±2.89	77.93^{a} ±7.69	62.7^{a} ±4.9	74.87^{a} ±3.84	123.33 ±9.29	90.03^{a} ±2.47	1.39^{e} ±0.01	1.6^{d} ±0.04	1.45^{d} ±0.09
T3	Yes (*Matrouh*)	Non	37.7^{c} ±4.09	54.83^{b} ±3.55	43.7^{c} ±3.15	65.97^{b} ±11.16	108 ±6.08	74.7^{cd} ±3.92	1.77^{a} ±0.1	1.98^{a} ±0.16	1.73^{a} ±0.05
T4	Yes (*Matrouh*)	Yes	37.4^{c} ±3.08	56.37^{b} ±5.61	45.1^{b} ±2.84	60.77^{bc} ±4.26	97.23 ±6.21	72.43^{d} ±3.16	1.64^{b} ±0.03	1.74^{b} ±0.1	1.62^{b} ±0.05
T5	Yes (*El-Behera*)	Non	43.03^{bc} ±1.56	64.97^{a} ±3.06	50.27^{b} ±3.98	65^{bc} ±1.93	109.33 ±2.89	79.3^{bc} ±2.7	1.52^{c} ±0.09	1.72^{c} ±0.12	1.6^{c} ±0.13
T6	Yes (*El-Behera*)	Yes	38.8^{c} ±1.61	68.3^{ab} ±5.41	48.17^{b} ±4.01	56.57^{c} ±7.84	106.27 ±15.11	77.67^{bcd} ±6.88	1.47^{d} ±0.26	1.56^{e} ±0.1	1.62^{b} ±0.08

*FCR: Feed Conversion Ratio.
Treatments within the same or no column with the same superscription letters were non significant ($P \leq 0.05$).

Table 2, indicated that maduramycin decreased oocyst count significantly (Log_{10}) in infected groups either with *Matrouh* (T4) or *El-Behera* isolates (T6) in comparison with infected-non medicated groups, respectively.

Maduramycin was effective in lesion control with *Matrouh* isolate (2.44 versus 2.78 for medicated group and non medicated one, respectively) , this results agreed with *(32)* while was not effective with El-Behera isolate in both groups (2.89).

Concerning mortality rates, both isolates either with or without maduramycin resulted in 3.3 % mortality. Previous investigation *(33)* recorded 1.67 % mortality among infected, medicated groups with maduramycin.

Although *El-Behera* isolate had less effect on weight gain, it had higher lesion scores, these result agreed with *(34)* who stated that there is no correlation between lesion scores and wieght gain.

It is clear that maduramycin reduced oocyst index and faecal scores (Table 3) of the birds infected with either *Matrouh* or *El-Behera* isolates than the birds which didn't receive the drug.

Regarding severity of coccidial infection with both E. tenella isolates, *Matrouh* isolate seemed to be more pathogenic than *El-Behera* isolate according to the results of weight gain and feed conversion ratio indecies.

The GIs of individual groups were given as a percentage of corresponding GI of NNC, where medicated groups with maduramycin that infected with either *Matrouh* or *El-Behera* isolates had GIs of 77.83 % and 81.51 % of NNC, respectively which indicated that maduramycin had limited efficacy against *Matrouh* isolate and good efficacy against *El-Behera* isolate. Weight gain % of infected (*Matrouh*), mediacted group was lower than that of infected (*El-Behera*), medicated ones

which support the idea that M*atrouh* isolate was more pathogenic than *El-Behera* isolate.

Resistance has been reported previously for maduramycin *(26,30)* and in this study, the efficacy of maduramycin ranged between limited (77.83% of NNC) to good (81.51% of NNC) and this may be due to intensive use of maduramycin or due to cross resistance between maduramycin and other ionophores *(35)*.

Table 2. Lesion scores, Oocyst index, Mortality % and Average oocyst count .

Treatment Number	Infection	Medication (maduramycin)	Lesion Scores	Oocyst index	Mortality %	Average Oocyst count / day (Log_{10})
T1	Non	Non	0	0	0	0
T2	Non	Yes	0	0	0	0
T3	Yes (*Matrouh*)	Non	$2.78^{a}\pm0.83$	++++	3.3	$4.62^{a}\pm0.59$
T4	Yes (*Matrouh*)	Yes	$2.44^{b}\pm0.73$	++	3.3	$4.45^{b}\pm0.57$
T5	Yes (*El-Behera*)	Non	$2.89^{a}\pm0.78$	++++	3.3	$5.03^{a}\pm0.24$
T6	Yes (*El-Behera*)	Yes	$2.89^{a}\pm0.98$	+++	3.3	$4.62^{b}\pm0.55$

Treatments within the same column with the same superscription letters were not significant ($P \leq 0.05$).

Oocyst index *(24)*

0: no oocyst / field. +1 : 1-10 oocysts / field. +2 : 11-20 oocysts / field.

+3 : 21-50 oocysts / field. +4 : 51-100 oocysts / field. +5 : > 100 oocysts / field.

Table 3. Faecal Score from 5th to 10th day post infection.

Treatment Number	Infection	Medication (maduramycin)	Faecal Score from 5th to 10th day post infection					
			5	6	7	8	9	10
T1	Non	Non	0	0	0	0	0	0
T2	Non	Yes	0	0	0	0	0	0
T3	Yes (*Matrouh*)	Non	++++	++++	++	+	+	+
T4	Yes (*Matrouh*)	Yes	++	+++	++	+	+	+
T5	Yes (*El-Behera*)	Non	++++	++++	+++	+	+	+
T6	Yes (*El-Behera*)	Yes	++	+++	++	+	+	+

Treatments within the same column with the same superscription letters were not significant ($P \leq 0.05$).

Faecal Score: *(25)*.

0: No bloody droppings in the faeces. +: 1-10 blood droppings in the faeces.

++: 10-20 blood droppings in the faeces. +++: 20-30 blood droppings in the faeces.

++++: 30-40 blood droppings in the faeces.

Exp. 2:Evaluation of the efficacy of diclazuril

From Table 4, It was shown that the dose of infection with *Matrouh* isolate(25x 10^3 sporulated oocysts / bird) was low as weight gain of NNC (T1) and infected (*Matrouh*), non medicated birds (T3) didn't differ significantly except at acute stage of infection (D0 to D+7 P.I)

According to ***(36)*** who indicated that when sensitive isolates were controlled by medication, weight gain responses are expected to be equivalent to NNC. Diclazuril was more effective on *Matrouh* isolate and this appeared from D0 to D+7 while at other testing periods there was no significant difference between the two treatments due to the light infection dose with *Matrouh* isolate and the battery reared chickens.

The lack of a significant difference in the weight gain of the infected groups with *El-Behera* isolate, non medicated (T5) and medicated groups (T6) and also with NNC was possibly due to the light infection dose, so the effect of diclazuril on *El-Behera* isolate was not obvious with this parameter.

Feed conversion ratio of all tested groups was significantly different except that of NNC and infected (*El-Behera*), non medicated group that were 1.4, 1.41, 1.47, 1.45, 1.4 and 1.37 among groups 1, 2, 3, 4, 5, and 6, respectively .

It was evident that diclazuril had good effect on both isolates. Also the light infection with *El-Behera* isolate was clear with FCR of infected (*El-Behera*), non medicated group in comparison to NNC.

Oocyst counts (Log_{10}) Table 5 were lower significantly in medicated groups that infected either with *Matrouh* (T4) or *El-Behera* isolates (T6) than that of non medicated–infected ones (T3) or (T5), respectively.

Mortality rates due to coccidiosis infection were only recorded in the infected groups with *Matrouh* isolate either medicated or unmedicated (3.3 %) versus 0 % in negative control group and group infected with *El-Behera* isolate.

No significant differences were observed between lesion scores of all tested groups either with or without treatment .

Although weight gain of infected birds with *El-Behera* isolate was not depressed, this group had lesion score of average 2.11 .There is no correlation between lesion scores and wight gain ***(34)***.

The results of oocyst counts were difficult to correlate with results of the coccidiosis lesion scores in both isolates which may be due to the occurrence of crowding effect ***(37,38).***

Oocyst index in birds infected with either *Matrouh* or *El-Behera* isolates and treated with diclazuril was lower than that of non medicated birds

Also it was clear that diclazuril reduced fecal scores of infected birds with *Matrouh* isolate, but didn't affect fecal scores with *El-Behera* isolate Table (6).

It was observed that *Matrouh* isolate was more pathogenic than *El-Behera* isolate through parameters of mortality %, lesion scores, faecal scores, oocyst index and oocyst count .

The GIs of individual groups were given as a percentage of corresponding GI of NNC, where medicated groups with diclazuril that infected with either *Matrouh* or *El-Behera* isolates had GIs of 84.54 % and 92.54 % of NNC, respectively which indicated that diclazuril had good efficacy against *Matrouh* isolate and very good effiecacy against *El-Behera* isolate, in spite of resistance which has been reported previously for diclazuril ***(37,39)***. This may be due to that the extent of drug usage and also due to the mode of action of diclazuril breaks down all intracellular developmental stages of the asexual and sexual reproductive cycles of *E. tenella* ***(40).***

Under the circumstances of this study, from Table 7, it could be concluded that maduramycin showed varying degrees of efficacy ranging from limited to good indicating that it's use should be restricted while diclazuril showed good to very good efficacy, so it's use should be handled thoroughly to keep it's efficacy in th field.

Table 4. Body gain, feed consumption and feed conversion ratio.

Treatment Number	Infection	Medication (diclazuril)	Body gain g / bird / day at		Average total gain g / bird / day at D0 to D+14	Feed consumption g / bird / day at		Average total feed consumption g / bird / day at D0 to D+14	FCR* / bird / day at		Average FCR / bird / day at D0 to D+14
			D0 to D+7	D+7 to D+14		D0 to D+7	D+7 to D+14		D0 to D+7	D+7 to D+14	
T1	Non	Non	48.63^{a} ±4.98	94.33 ±5.44	62.5 ±1.11	80.57 ±3.00	119.43 ±5.42	87.5 ±1.00	1.67^{d} ±0.24	1.27^{c} ±0.08	1.4^{d} ±0.02
T2	Non	Yes	46.4^{ab} ±3.73	82.83 ±10.83	58.83 ±3.53	73.6 ±4.98	111.57 ±19.07	83.07 ±7.54	1.59^{e} ±0.04	1.34^{b} ±0.05	1.41^{c} ±0.05
T3	Yes (*Matrouh*)	Non	37.33^{b} ±0.21	82.37 ±12.47	53.67 ±4.39	66.8 ±3.67	104.53 ±18.25	78.77 ±6.12	1.79^{a} ±0.10	1.27^{c} ±0.07	1.47^{a} ±0.04
T4	Yes (*Matrouh*)	Yes	40.2^{ab} ±7.55	78.17 7.65	53.37 ±5.49	70.6 ±10.97	96.87 ±13.75	77.17 ±8.87	1.76^{b} ±0.09	1.24^{e} ±0.07	1.45^{b} ±0.08
T5	Yes (*El-Behera*)	Non	48.3^{ab} ±1.87	89.67 ±2.17	62.53 ±1.54	76.33 ±1.55	127.13 ±3.76	87.8 ±3.51	1.58^{e} ±0.04	1.42^{a} ±0.07	1.4^{d} ±0.03
T6	Yes (*El-Behera*)	Yes	42.07^{ab} ±7.63	84.87 ±4.08	56.97 ±7.32	71.1 ±10.19	106.3 ±10.81	77.7 ±6.6	1.71^{c} ±0.26	1.25^{d} ±0.07	1.37^{e} ±0.09

*FCR: Feed Conversion ratio.

Treatments within the same or no column with the same superscription letters were non significant ($P \leq 0.05$).

Table 5. Lesion scores, Oocyst index, Mortality % and Average oocyst count.

Treatment Number	Infection	Medication (diclazuril)	Lesion Scores	Oocyst index	Mortality %	Average oocyst count / day (Log_{10})
T1	Non	Non	0	0	0	0
T2	Non	Yes	0	0	0	0
T3	Yes (*Matrouh*)	Non	2.33 ±0.5	++++	3.3	4.82 ± 0.54
T4	Yes (*Matrouh*)	Yes	2.22 ±0.8	+++	3.3	4.72 ±0.59
T5	Yes (*El-Behera*)	Non	2.11 ±1.05	+++	0	4.8 ±0.45
T6	Yes (*El-Behera*)	Yes	2.11 ±0.78	++	0	4.63 ±0.55

Treatments within the same column with the same superscription letters show no significant ($P \leq 0.05$).

Oocyst index *(24)*.

0: No oocyst / field. +1 : 1-10 oocysts / field. +2 : 11-20 oocysts / field.

+3 : 21-50 oocysts / field. +4 : 51-100 oocysts / field. +5 : > 100 oocysts / field.

Table 6. Faecal Score from 5th to 10th day post infection.

Treatment Number	Infection	Medication (diclazuril)	Faecal score from 5th to 10th day post infection					
			5	6	7	8	9	10
T1	Non	Non	0	0	0	0	0	0
T2	Non	Yes	0	0	0	0	0	0
T3	Yes (*Matrouh*)	Non	+++	+++	+	++	++	+
T4	Yes (*Matrouh*)	Yes	++	++	+	++	+	+
T5	Yes (*El-Behera*)	Non	+	++	+	++	+	+
T6	Yes (*El-Behera*)	Yes	+	++	+	+	+	+

Treatments within the same or no column with the same superscription letters were non significant ($P \leq 0.05$).
Faecal Score: *(25)*
0: No blood droppings in the faeces.
+: 1-10 blood droppings in the faeces.
++: 10-20 blood droppings in the faeces.
+++: 20-30 blood droppings in the faeces.
++++: 30-40 blood droppings in the faeces.

Table 7. Global Index (GIs) of the individual study groups as a percentage of the corresponding GI of the NNC

Medication	GI % NNC			
	Matrouh isolate		*El-Behera* isolate	
	GI %	Judgement	GI %	Judgement
Maduramycin	77.83	Limited efficacy	81.51	Good efficacy
Diclazuril	85.05	Good efficacy	92.54	Very good efficacy

NNC: Non infected/Non medicated Control.
GI: Global Resistance Index.

ACKNOWLEDGEMENT

The authors thank Dr A.M.Awad for providing the two isolates of *E. tenella*, Prof. Dr Y. A. Atia for statistical analysis also thanks all workers of Poultry and fish diseases departement, Fac.Vet.Med, Alex. Univ. for great help on this work.

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الملخص العربى

حساسية معزولتين من الايمريا تينيلا للماديورا ميسين والداى كلازوريل

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تم إجراء تجربتين فى بطاريات كل منها مكونة من سته معاملات بكل معاملة ثلاث مكررات وبكل مكررة عشرة طيور لدراسة كفاءة الماديورا ميسين والداى كلازوريل على معزولتين من الايمريا تينيلا التى عزلت من الحقل من محافظتى مطروح والبحيرة ٠

تم أعطاء الماديورا ميسين والداى كلازوريل فى العليقة للطيور بتركيز خمسة جزء فى المليون وواحد جزء فى المليون على التوالى بداية من يومين قبل العدوى وحتى نهاية التجربة (أسبوعين بعد العدوى) وتم عدوى الطيور ب ٢٥ × ١٠ ^{٣} حويصلة بالفم لكل من المعزولتين عند عمر أسبوعين٠

تم ملاحظة المردود الاقتصادى للطيور فى التجارب خلال ١٤ يوم بعد العدوى وبحساب الـ Global resistance index وجد أن كفاءة الماديورا ميسين لهذا الاختبار تتراوح بين ٧٧,٨٣% و ٨٥,٠٥% لكل من المعزولات المعزولة من مطروح والبحيرة على التوالى ٠ بينما كفاءة الداى كلازوريل تتراوحبين ٨١,٥١% و ٩٢,٥٤% ٠