ANTICOCCIDIAL EFFICACY OF METRONIDAZOLE

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ABSTRACT

This study was carried out to study the efficacy of metronidazole as anticoccidial drug at two levels of doses (25 and 50 mg / kg B.W) for 5 consecutive days via drinking water. One hundred and fifty mixed sex one day old Arbor Acres F.s. chicks were divided into six equal main groups. All were fed on ordinary ration free from any anticoccidial agents allover the experiment (41 days). The first group was non infected non treated (negative control). the second group was treated without infection with 25 mg / kg of metronidazole (flagyl susp Alex co) ,the third group was treated with 50 mg/kg B.W of metronidazole without infection. The fourth group was infected non treated group (positive control) .The fifth and sixth groups were infected with E. tenella (50,000 oocysts / bird) at 18 days of age and treated with( 25 and 50mg /kg B.W) of metronidazole respectively after symptoms appeared for 5 consecutive days .

The evaluation was based on clinical signs, lesion scores, oocyst counts, and histopathological findings of treated and infected non treated group. The results showed that the infected non treated group (positive control) showed the typical signs of coccidiosis including depression, loss of appetite and bloody droppings one week post infection. Variable degrees of illness were recorded until the end of experiment.

Medication of metronidazole after infection at both dose levels improved the clinical signs, oocyst counts and Lesion score,The mortality rate as well as the epithelial lining of the intestinal gland of infected treated group than that of the infected non treated group, in addition to the degeneration of most of the developmental stages of E tenella in treated Groups.
INTRODUCTION

Coccidiosis remains one of the most expensive and common-diseases of poultry inspite of advances in chemotherapy, management, nutrition and genetics. (Conway and Mckenzei, 1991 and Mc Dougald and Reid, 1991).

The development of resistance to anticoccidial drugs is a major problem in poultry industry. Anticoccidial drug resistance occurs when eimeria can multiply or survive in the presence of a concentration of an anticoccidial drug that normally destroy parasites of the same species or prevent their multiplication (Chapman 1997). This Resistance and Cross-resistance against coccidiostats continuously motivates for the search for new agents of anticoccidial activity.

The isolation of the antibiotic azomycin (2- nitroimidazole) from a Streptomycete and demonstration of its trichomonacidal properties led to the chemical synthesis and biological testing of many nitroimidazoles. One compound, 1- (B-hydroxyethyl), 2-methyl- 5 nitroimidazol, now called metronidazole (Flagyl and others), was very active against T, vaginalis infections in genitourinary tract in both male and female (Cosar and Julou, 1959, Durcl et al., 1959; Cosonka; 1971, Thin et al; 1979; Hager et al 1980), Tricmoniasis in pigeons (Yang et al.1988, Abd El Motelib and Galal, 1994, Aydin et al.2000); against Tricmoniasis in geese (Ziomko et al.1991) against Giardia lamblia (Fowler, 1960; Schneider, 1961 and Boreham et al. 1984), against intestinal and liver ameabiasis (Pehrson and Bengtsson, 1984 and Cosar et al.1961) treatment of Black head disease (Histomoniasis) (Mc dougald et al 1988, Muller;1990 and Hegngi et al 1999).
Some authors recorded that metronidazole was effective against hepatic and intestinal coccidiosis in rabbits (Reshetnyak 
et al;1960, Jones et al., 1977 and Zhang and Xue, 1990), coccidiosis in calves (Alfonso et al. .1982) and E tenella in chickens (Shakslioiik et al.1995).

From the available literatures there were a few records about the anticoccidial efficacy of metronidazole so the present study aimed to evaluate the effect of metronidazole against ceecal coccidiosis in chickens on the bases of clinical signs, lesion scores, oocyst counts, bird performance and histopathological findings in the Ceaci of treated and infected non treated groups.

**MATERIALS AND METHODS**

I- Grouping and experimental design:

One hundred and fifty mixed sex one day old Arbor Acres Fs chicks were divided into 4 main equal groups, All chicks were fed on ordinary ration (standard broiler ration from El-Roda company) free from any anticoccidial drugs all over the experiment (41 days). The first group was kept non infected and served as the negative control, the second group was treated without infection with 25 mg / kg of metronidazole (flagyl susp Alex co), the third group was treated with 50 mg/kg B.W of metronidazole without infection the fourth group was infected with E. tenella (50.000 oocysts / bird) at 18 days of age and considered as a positive non treated control group. The fifth group was infected with E. tenella (50.000 oocysts / bird) at 18 days of age and treated with 25 mg kg B.W. of metronidazole,. The drug was given for 5 successive days after symptoms appeared (day 23 to day 27) and the sixth group was infected
with _E. tenella_ (50,000 oocysts / bird) at 18 days of age and treated with 50 mg/Kg B.W. of metronidazole. The drug was given for 5 successive days after symptoms appeared (day 23 to day 27). The oocysts were mixed with 50 grams of feed per bird and the birds were fastened for 2 hours, then fed on these 50 grams and no other experimental ration was provided until all the contaminated feed was consumed.

**II- Preparation of _E. tenella_ inoculum and experimental infection:**

1. Isolation of field _E. tenella_ strain, sporulation and propagation of their oocysts:

   Ceaci of infected chickens from a commercial broiler farm were obtained. The ceecal contents were homogenized with water and sieved in a beaker through a fine wire mesh. The filtrate was let to sediment. The supernatant fluid was discarded and the pellet was resuspended in potassium dichromate 2.5% in a group of Petri dishes. The thickness of fluid was not higher than 5 mm to facilitate the oxygen diffusion. Forced aeration was achieved (2- 3 times daily). After sporulation the sporulated oocysts were removed from the fecal debris by a series of centrifugations using NaCl (concentration flotation technique). The suspension was centrifuged at a moderate speed (1500' rpm) for 5-10 minutes to sediment the solids and allow the oocysts to suspend at the top of the supernatant. The floated oocysts were collected by Pasteur pipette and propagated in ten 15-days old coccidian free chicks which were housed in batteries, fed on coccidiostat free ration and at day 18 of age, infected with 20,000 oocysts per bird by direct inoculation into the crop using rubber tubing. On the 5lh day post infection, feed was withdrawn and the trays under the cages were cleaned out and about 1-5 liters of potassium dichromate
2.5% were put in the trays. The oocysts shedding began on the day 6 post infection. On day 8 after infection all birds were slaughtered and the 3 days fecal collections with the ceacal contents of the killed birds were collected for separation of large volume of oocysts these oocysts were sporulated in potassium dichromate 2.5% as mentioned above.

2. Determination of the pathogenicity of the used strain and the optimal number of oocysts needed for infection for each bird:

Groups of four birds were housed in batteries and given different levels of oocysts mixed with their feed. 20000, 40000, 50000 and 70000 oocysts per each bird in the first, second, third and fourth group respectively. Infection was carried out 18 days of age. The selected dose of oocysts depends on the most adversely affected general state of health, droppings changes, lesion scoring and average mortality. From the test, 50000 oocysts per bird were found to be the most suitable for future use in the test (the most affected general state of health with average mortality).

III- Sampling:

1- Fecal samples:

Representative fresh litter samples were collected daily from the 6th day after infection (day23 of age) until 2 weeks post infection (day35 age).

2 -Sampling for histopatholgical examination:

Specimens from ceacum were collected from all groups and fixed in 10% buffered neutral formalin, until processed and stained (Lillie, 1954).
VI- Evaluation of the anticoccidial efficacy of metronidazole:

1- Clinical signs:

After infection, chickens were kept under observation for recording the intensity of clinical signs of coccidiosis as diarrhea, bloody feces, stop feeding and depression (Brandcr et al., 1991).

2- Oocyst count: The oocyst count was carried out according to the method described by Abd El-Rahman et al., (1982).

3- Post mortem and lesion score:

The method described by Johnson and Reid, (1970) was used. Five birds were killed one week post infection and a scoring system was adopted between 0 and ++++.

The feces of chickens were examined daily from day 6 of age to insure that there was no external infection with coccidiosis. The ceaci were examined to insure that the produced lesions were from coccidiosis.

V- Statistical analysis:

Data obtained were statistically analyzed using Students "t" test according to Snedecor and Cochran, (1967).

RESULTS

A- Anticoccidial efficacy of metronidazole:

1- Clinical signs:

The infected non treated group(4) showed depression, loss of appetite, anorexia, ruffled feathers and intensive bloody diarrhea one week after infection. These signs were more severe by the 14th day post infection (fig. 1). The signs subsided gradually with observation of few discolored droppings and varying degrees of depression until the end of the experiment.
In the group infected and treated with 25 mg/kg metronidazole, chickens were apparently normal but in the first week there was a mild depression and slight discoloration of feaces. One week post treatment this group was apparently normal (Fig.2). Clinically there were no noticeable differences between group infected and treated 25 mg/kg body weight and the group infected and treated with 50 mg/ kg B.W. of metronidazole .

There were no noticeable differences between the treated groups 2 and 3 without infection and the negative control group (group 1).

2- Post mortem and Lesion scoring:

The Ceaci of infected non treated group were dilated, their mucous membrane assumed a red colour easily visible even on the surface of the serrosa, and contain clotted blood (lesion score ++++) (Fig. 3-B and 4) while the groups treated with 25 and 50 mg metronidazole /k.g B.w. showed slight thickening of ceacal wall, with bloody lesions in the cecal wall (lesion score ++) Fig.3-A and 3- C).

3- Mortality rate:

The infected non treated group showed a significant high mortality rate (24% while the mortality rate of all treated groups were significantly decreased to 4 % (Table 1).

4- Oocysts counting:

The infected non treated control group showed the highest oocyst counting allover the days of the counting. The oocyst counting of all treated groups (25 and 50mg/kg B.W) of metronidazole was significantly decreased in comparison with the infected non treated group (Table 2), and there were no apparent differences between the group treated with 25 mg /Kg B.W and group treated with 50 mg /K.g B.W of metronidazole.
5- Histopathological Findings:

In the 1st week post infection, the control infected non treated group showed severe desquamation of epithelial lining of the ceacum with massive leukocytic infiltration in lamina propria (Fig 5), intensive colonization of lamina propria with different developmental stages specially schizonts (Fig. 7), and In the last week of experiment, this group showed extensive leukocytic infiltration with necrosis and complete disappearance of the lining epithelium of intestinal gland (Fig 9).

The infected treated group (25 mg/kg body weight) showed slight desquamation of epithelial lining with leukocytic infiltration in Lamina propria and there were hyperplasia of the epithelial lining of intestinal gland. (Fig. 6), and a few developmental stages of E. tenella with varying degrees of degeneration (Fig 8). The two groups given metronidazole after infection showed hyperplasia of the epithelial lining of intestinal glands (fig 10).

**Table (1):** The number of dead birds per week and mortality rate under treatment *E.tenellu* infected chickens by metronidazole.

<table>
<thead>
<tr>
<th>Groups Age post infection</th>
<th>Group (1) Control -ve</th>
<th>Group (2) Treated w/ 25 mg/kg B.W</th>
<th>Group (3) Treated w/ 50 mg/kg B.W</th>
<th>Group (4) Control-ve</th>
<th>Group (5) Treated after infection with 25 mg/Kg B.W</th>
<th>Group (6) Treated after infection with 50 mg/kg B.W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd week</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3rd week</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total number</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mortality rate%</td>
<td>_</td>
<td>%4</td>
<td>%4</td>
<td>%24</td>
<td>%4</td>
<td>%4</td>
</tr>
</tbody>
</table>

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**Table (2):** The effect of metronidazole given as therapeutic treatment on oocyst count (x 10-3)/gm feces in chicks infected n=5.

<table>
<thead>
<tr>
<th>Groups/ Age in days</th>
<th>Group (4) Control +ve</th>
<th>Group (5) Treated with 25mg/kg</th>
<th>Group (6) Treated with 50mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>128±1.5</td>
<td>112*** ± 1.6</td>
<td>105.6*** ±0.6</td>
</tr>
<tr>
<td>25</td>
<td>320 ±0.31</td>
<td>220.8*** ± 1.31</td>
<td>153.6*** ±1.3</td>
</tr>
<tr>
<td>26</td>
<td>502.4 ±0.599</td>
<td>211.2*** ± 1.390</td>
<td>214.4*** ±0.286</td>
</tr>
<tr>
<td>27</td>
<td>272 ±0.315</td>
<td>172.8***± 1.316</td>
<td>73.6*** ± 1.286</td>
</tr>
<tr>
<td>28</td>
<td>96 ± 1.480</td>
<td>80***± 1.578</td>
<td>105.6***± 1.286</td>
</tr>
<tr>
<td>29</td>
<td>90.72 ±16.180</td>
<td>45.8* ± 1.494</td>
<td>46.2* ± 1.278</td>
</tr>
<tr>
<td>30</td>
<td>23.52 ± 16.184</td>
<td>24.12 ± 1.909</td>
<td>24.16*±2.196</td>
</tr>
<tr>
<td>31</td>
<td>9.16 ± 1.065</td>
<td>3.84**± 0.5820</td>
<td>3.08** ± 1.432</td>
</tr>
<tr>
<td>32</td>
<td>7.16 ± 1.238</td>
<td>3.53- ± 1.453</td>
<td>2.73* ±2.33 ± 2.33</td>
</tr>
<tr>
<td>33</td>
<td>6.96 ± 1.1978</td>
<td>3.16*±1.0658</td>
<td>2.12*±2.1544</td>
</tr>
<tr>
<td>34</td>
<td>6.6 ± 0.9257</td>
<td>1.6*±1.9201</td>
<td>1.8* ±0.5090</td>
</tr>
<tr>
<td>35</td>
<td>4.8 ± 1.921</td>
<td>1.32* ±1.0277</td>
<td>1.6*±3.076 ±3.076</td>
</tr>
</tbody>
</table>

* Significant at (P < 0.05) compared with control +ve.

** LIST OF FIGURES **

**Fig. (1):** Showing depression, loss of appetite, ruffled feathers in infected none treated chickens (+ve control group) by the 7th day post infection.

**Fig (2):** showing apparently normal chickens previously infected and treated with 25mg/kg body weight of metronidazole.

**Fig. (3):** Showing lesion scores in the ceaci of infected treated and infected non treated chickens.

A= The ceaci of chickens treated with 25 mg/kg of metronidazole (score ++).

B= The ceaci of the control +ve infected non treated group (score +++).

C= The ceaci of chickens treated with 50 mg/kg of metronidazole (++).

Fig. (4): Showing the ceca of infected non treated group dilated with clotted blood and severe hemorrhage in the dead birds.

Fig. (5): Section in the cecum of infected non treated control group showing severe desquamation of epithelial lining and leukocytic infiltration in Lamina propria H & E, (XI00).

Fig. (6): Section in the cecum of infected group treated with 25 mg/kg body weight at 32nd day showing slight desquamation of epithelial lining and leukocytic infiltration in lamina propria and hyperplasia of epithelial lining of intestinal glands. H & E, (X 200).

Fig. (7): Section in the cecum of infected non treated control group showing different developmental stages of E. tenella in the epithelial lining and lamina propria. H & E (X 400)

Fig. (8): Section in the cecum of infected group treated with 25 mg/kg body weight at 32nd day showing different developmental stages of Eimeria with different degrees of degeneration H & E (X 4000).

Fig. (9): Section in the cecum of infected chicken none treated at 39th day of age showing extensive leukocytic infiltration of the whole thickness of intestinal wall together with necrosis and completes disappearance of epithelial lining of intestinal glands. H & E (XI00).

Fig. (10): Section in the cecum of infected chicken treated with metronidazole 25 mg/kg BW showing hyperplasia of lining epithelial of intestinal glands and edema. H & E (X 100)
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1. Image 1
2. Image 2
3. Image 3
4. Image 4
5. Image 5
6. Image 6
7. Image 7
8. Image 8
9. Image 9
10. Image 10


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DISCUSSION

The results of the present investigation revealed the incidence of clinical manifestations in chickens infested by Eimeria tenella oocysts and none treated, represented by depression, Loss of appetite and bloody droppings. Such findings are evaluated as common signs of cecal coccidiosis (Hofstad et al., 1984).

Chickens given metronidazole at a dose level of 25 mg / kg body weight for five consecutive days, showed mild depression and slight discoloration of droppings which was improved by the end of 1st week post infection. However, chickens given 50 mg /kg of metronidazole were almost apparently normal. This indicates the usefulness of using metronidazole at a dose level 25mg / kg body weight for treatment of coccidiosis in chickens. These findings were in agreement with results achieved by Reshetnyak et al., (1970) and Shakshouk et al., (1995) who reported that metronidazole was fully effective in preventing lesions due to cecal coccidiosis and reduced clinical signs by 40 mg / kg body weight for 3 days in rabbits and by 25 mg /kg body weight in chickens. The possible explanation of the previous notion in that the need for an increased dose in rabbits may be due to difference in species capability of absorption of the drug or it might be due to genetic variability in the activity of metabolizing hepatic microsomal enzymes.

In the present study, it has been shown that the oocyst count was significantly reduced by the use of metronidazole( 25 mg / kg body weight) and there was no significant difference in reduction by using metronidazole at 50 mg / kg body weight in comparison with the chickens given 25mg / kg body weight of metronidazole. This indicates the usefulness of using metronidazole at a dose level of 25mg / kg body weight. Similar results were recorded by Alfanso et al., (1982) and Shakshouk et al., (1995). They reported that metronidazole (25 mg / kg body weight) was effective in reducing the oocyst
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shedding and lesion scores of cecal coccidiosis. Also, these results coincide with Zhang and Xue (1990) they reported that the liver lesion scores and average oocyst counts in rabbits treated by metronidazole were lower than non treated groups.

Coccidiosis induces a high percentage of morbidity rather than mortality rate. However, the mortality rate of the infected non treated group in the present study was 24%. It was a good achievement that the use of metronidazole either in a dose level of 25 mg or 50 mg / kg body weight in chickens infected with E.tenella regressed the mortality rate to be 4 %. Retardation of mortality in infected chickens or calves treated with metronidazole to a level of 5% was also an achievement recorded respectively by Shakshouk et al., (1995) and Alfonso et al., (1982).

Regarding the histopathological findings in relation to examined ceaci, the infected non treated group showed the epithelial lining and lamina propria of the ceaci were colonized with a great numbers of different developmental stages of E.tenella accompanied with desquamation and sloughing of the epithelial mucosa. These findings were also recorded by Waletzky et al., (1945), Witlock et al., (1975), Kimura et al., (1975) and Fernardo ct al., (1983).

The histopatholgical exmaination of samples collected from the infected groups treated by variable doses of metronidazole revealed the perfect control of infection and almost absence of any possible adverse effects to be recorded.

However, ceaci from chickens given therapeutic doses of metronidazole (25, 50 mg / kg body weight) showed few developmental stages of Eimeria in the cecal wall. These few developmental stages showed varying degrees of degeneration accompanied with a slight desquamation of the epithelial cells with hyperplasia of the intestinal glands and leukocytic infiltration in lamina propria, as compared with the pathological findings in the infected non treated group. These results are confirmative for a successful control of coccidiosis.
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تأثير عقار الميترونيديازول على تطفيل الكوكسديا

مصطفى عبد العزيز ، ظاهر عبد الوهاب ، كمال الشاذلي ، على صلاح سيف

أجريت هذه الدراسة على عدد 150 كتَنَكَت بدارى تسمين من سلالة أوبوايكرز أفي أس تسمين والتي تم تغذيتها على علاجات خالية من مضادات الكوكسديا من عمر يوم وحتى نهاية التجربة وقد قسمت الكتَنَكَت إلى 6 مجموعات لكل مجموعة 25 كتَنَكَت المجموعة الأولى ضابطة سلبية المجموعات الثانية والثالثة تلقفت علاج بجرعات 25 و 50 مجم ميترونيديازول لكل كجم من وزن الطائر على التوالي لمدة خمسة أيام متتالية وبعدين عدى بالكوكسديا، المجموعة الرابعة أعطيت عدوى بالكوكسديا 50000 حويصلة من طفيل الأمبريا تتبلا لكل طائر وبعد علاج إيجابي. المجموعة الخامسة والسادسة أعطيت عدوى بالكوكسديا 50000 حويصلة من طفيل الأمبريا تتبلا لكل طائر ثم عولجت بعد ظهور الأعراض عند عمر 23 يوم بجرعات علاجية 25 و 50 مجم ميترونيديازول لكل كجم من وزن الطائر للمجموعة الخامسة والسادسة على التوالي لمتخصصة أيام متتالية تمت الملاحظة الإكلينيكية للكتَنَكَت طوال فترة التجربة وأجريت الفحوص التشخيصية المرضية الأسبوعيا عند عمر 39،32،25، وكذلك العد اليومي لبويضات الطفيل.

وقد تبين أن العلاج بالميترونيديازول سواء بالجرعة 25 أو 50 مجم ميترونيديازول لكل كجم من وزن الطائر أحدث انخفاض معنوي في عدد حوياصلات الكوكسديا في البراز كذلك انخفاض في معدل النفوذ والآفات التشريحية المرضية بالإضافة إلى تدمير معظم أطوار النمو المختلفة لطفيل الأمبريا تتبلا داخل الخلايا المبطنة لللاعرين.