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EVALUATION THE RESISTENCE OF *EIMERIA SPP.* LOCAL ISOLATES TO ANTICCOCIDIAL DRUGS AND THE EFFICACY OF LIVE ATTENUATED VACCINE AND/OR PREBIOTIC IN CONTROL OF EIMERIA INFECTION IN FAYOUMI CHICKENS

HASSAN, AHMED [2] ; MAHMOUD, WARDAA [1]; SHEHATA, MOSTAFA [2] AND ARAFA, MOHSEN [3]

[1] Animal Health Research Institute, El-Menia Branch, El-Menia, Egypt

[2] Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

[3] Dept. of Parasitology, Animal Health Research Institute, Assiut Lab.

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ABSTRACT

Coccidiosis is the most important intestinal parasitic disease of poultry worldwide. It may lead to high economic losses in poultry because of the high mortality rates and high cost of medication. The present study aimed to evaluate the resistance of *Eimeria* spices local isolates to anticoccidials and to study the efficacy of live attenuated vaccines and/or prebiotics in control of *Eimeria* infection in Fayoumi chickens. Seventy gut samples of Faioumy chickens at 29-39 days old suspected for coccidiosis infection were collected from the research station of animal production at Malawi city, El-Minia Governorate, and subjected to isolation and morphological identification. Resistance of *Eimeria* species to anticoccidial drugs was evaluated using anticoccidial sensitivity profiles 1 (ASP1) and 2 (ASP2). Evaluation of the effectiveness of living attenuated vaccine (Fortegra) and/or prebiotic (Agrimos) in control of coccidiosis was done using experimental challenge with isolated Eimeria species. It was concluded that using morphological methods for identification of Eimeria spp. in Fayoumi chickens, five *Eimeria* spp. could be isolated; *E. mitis, E. maxima, E. acervuline, E. tenella,* and *E.* praecox. The present study provides evidence for the resistance of isolated *Eimeria* spp. to toltrazuril and good sensitivity to sulfaclozine and amprolium in fayoumi chickens. This study showed that living attenuated vaccine is effective for control of coccidiosis and better results will be obtained in case of addition of prebiotic to vaccine. The use of prebiotics in control of Eimeria infection is not effective.

Key words: Coccidiosis, Vaccine, Prebiotic, Control, Fayoumi chickens.

INTRODUCTION

Poultry industry is not an important source of animal protein only but also plays an important role in the employment. This organized and essential industry is adversely affected by very important diseases of poultry that worldwide spread (Lee *et al*., 2009). The cause of this disease is apicomplexan parasites of the genus *Eimeria,* which causes coccidiosis. In domestic birds, nine species of *Eimeria* have been identified (Morgan *et al*., 2009). *Eimeria tenella* and *Eimeria necatrix* are the most pathogenic; *Eimeria acervuline*, *Eimeria maxima*, and *Eimeria mivati* are

Corresponding author: Hassan, Ahmed
E-mail address: a.hassan@aun.edu.eg
Present address: Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

common and moderately pathogenic; *E. brunetti* is less common but pathogenic when it occurs, while *Eimeria praecox*, *Eimeria mitis,* and *Eimeria hagani* are relatively nonpathogenic species (Soulsby, 1982, Jadhav *et al*., 2011). Coccidiosis is characterized by variable types of clinical signs, such as enteritis, dysentery, diarrhea, which may be sometimes bloody with some *Eimeria species*, emaciation, low feed conversion rate, delayed sexual maturation, droopy wings, poor growth, lower production, and even death (Rehman *et al*., 2011; Awais *et al*., 2012).

Despite advanced methods of immunology, biotechnology, and genetics, control of coccidial infection mainly depends on prophylactic chemotherapeutic anticoccidials (McDougald and Reid, 1997). However, the drug resistance in coccidia is an important problem with most drugs, due to the course limit of their use. Drug resistance in coccidiosis has been reported against most anticoccidial drugs that have been used (Abbas *et al*., 2011).

Eimeria drug resistance still remains an enormous obstacle. Resistance of many *Eimeria* spp. isolates to sulfaquinoxaline, amprolium, nitrofurazone plus furazolidone, nicarbazin, clopidol, sodium sulphadimethyl pyrimidine and maduramicin have been reported (Agarwal *et al*., 2013). Since *Eimeria spp*. are highly immunogenic and can stimulate solid immunity to homologous challenge, immunological control can serve as a practical alternative to indiscriminate use of anticoccidials. Based on this concept, a number of live or live attenuated vaccines have been marketed globally.

Efficacy of vaccination is variable and may be restricted in some circumstances, which might need development of new protocols that could improve vaccine effectiveness (Allen *et al*., 2004). In fact, improvement of immunity through good hygiene practices and use of feed additives could be a significant complementary approach (Vermeulen *et al*., 2001). Many strategies could be alternative solutions that proved their efficacy in control of coccidiosis, with significant positive effects on immune status and performance of birds. These protocols depend mainly on the maintenance of the intestinal barrier integrity and the birds' immune response stimulation (Elmusharaf *et al*., 2007; Gomez-Verduzco *et al*., 2009; Oliviera *et al*., 2009; Swiatkiewicz *et al*., 2014). Thus, it is important to use feed additives to prevent diseases such as coccidiosis and improve the utilization of nutrients.

The current study aimed to evaluate the resistance of *Eimeria* spp. local isolates to common anticoccidials and to study the efficacy of live attenuated vaccine and/or prebiotics in control of Eimeria infection in Fayoumi chickens.

MATERIALS AND METHODS

1. Samples collection and identification of *Eimeria species*:

A total of 70 gut samples of Faioumy chickens at 29 - 39 days old suspected for coccidiosis infection were collected from the research station of animal production at Malawi City, Minia Governorate during the period from February to May 2021. Each sample was collected separately in a plastic bag and transported to the laboratory for examination by direct smear method. For isolation of the oocysts of Eimeria species, sedimentation technique (Soulsby, 1986) was used. The sporulation of the oocysts was done according to Reid and Long, 1979 for confirmation of Eimeria species. The identification of *Eimeria* spp. was depends upon oocysts and sporocysts morphology like; color, shape, form index, micropyle and its cap, and absence or presence of residual, and time of sporulation. Oocyst shape index was done according to Edgar and Siebold, 1964.

2. Experimental infection:

2.1. Anticoccidial drugs, probiotic, and vaccine:

Anticoccidial drugs including toltrazuril (Toltavet 2.5% oral solution, Mobedo-vet company, Jordan), amprolium (Amprosol 60% oral solution, Delta-vet company, Egypt), diclazuril (Diclazomix 1% oral solution, Amon-vet company, Egypt), and sulfaclozine (Clozicocc 32% powder, Pharco company, Egypt) were used for experimental infection. Prebiotic (Agrimos) obtained from Lallemand inc company was used in the present experiment. A living attenuated vaccine (Fortegra, MSD Animal Health Inc company) contains live oocysts of *Eimeria acervulina, E. mivati, E. maxima, E. maxima, and E. tenella* was used.

2.2. Preparation of infective inoculum:

Sporulated oocysts were washed with distilled water three times, and then were counted using the McMaster counting technique, which used to quantify the number of sporulated oocysts per ml of suspension (Ryley *et al*., 1976).

2.3. Experimental design:

Two hundreds and seventy, one-day-old, Faioumy chicks were obtained from a local hatchery (Research Station of Animal Production in Malawi). Pooled fecal swabs were collected from chicks and then examined for parasitic infections. Chicks were reared under complete sanitary conditions. All the chicks were fed balanced commercial rations free from anticoccidial drugs, and the feed and water were given ad libitum. Chicks were divided randomly into nine equal groups; each group contains thirty chicks. Experimental groups were designed as the following: group (1), chicks were vaccinated with the Living (Fortegra) vaccine at 1st day of age and experimentally infected with 1 x 10^5 cecal *Eimeria spp.* sporulated oocyst at 14th days of age; group (2), chicks were treated with prebiotic (Agrimos) from 1st day of age till the end of the experiment, and experimentally infected with 1 x 10^5 mixed *Eimeria spp.* sporulated oocyst at 14th days of age, group (3); chicks were vaccinated with Fortegra vaccine at 1st day of age and treated with prebiotic (Agrimos) from 1st day of age till the end of the experiment, and experimentally infected with 1 x 10^5 cecal *Eimeria spp.* sporulated oocyst at 14th days of age; group (4), chicks were treated with sulfaclozine (Clozicocc) at 12th day of age and continued for 5 successive days, and experimentally infected with 1 x 10^5 mixed *Eimeria spp.* sporulated oocyst at 14th days of age; group (5), chicks were treated with amprolium (Aprosol) at 12th day of age and continued for 7 successive days and experimentally infected with 1 x 10^5 mixed *Eimeria spp.* sporulated oocyst at 14th days of age; group (6), chicks were treated with diclazuril (Diclazomix) at 12th day of age and continued for 3 successive days and experimentally infected with 1 x 10^5 mixed *Eimeria spp.* sporulated oocyst at 14th days of age; group (7), chicks were treated with toltrazuril (Toltavet) at 12th day of age and continued for 2 successive days and experimentally infected with 1 x 10^5 mixed *Eimeria spp.* sporulated oocyst at 14th days of age; group (8), chicks were kept as negative control, not infected and not treated; and group (8), chicks were kept as positive control, infected and not treated.

3. Evaluation the resistance of Eimeria spp. to anticoccidial drugs:

3.1. Anticoccidial sensitivity profile 1 (ASP1):

Mean lesion scores (MLS) between non-medicated *Eimeria* infected groups and medicated *Eimeria* infected groups were compared using the method described by McDougald *et al*.,1986, who use the following formula:

100% - (MLS of medicated-infected group /MLS of non-medicated-infected group x100%). Results of 0-30% indicate resistance, results of 31-49% indicate partial resistance, results >50 % indicate full sensitivity.

3.2. Anticoccidial sensitivity profile 2 (ASP1):

Uses an anticoccidial index (ACI). Anticoccidial index was calculated by using the formula according to Shah *et al*., 2009 and Ma *et al.*, 2011.

ACI = (relative rate of weight gain + survival rate) - (lesion value (MLS x 10) + oocyst value (mean number of oocysts /10^6) x 0.4). *Eimeria* isolates were considered sensitive if the ACI > 160, partial resistant when the ACI was 120-160, complete resistant when the ACI < 120.

4. Evaluation of the efficacy of vaccines and/or prebiotics on control of Eimeria spp. infection using:

4.1. Faecal (Dropping) score:

Fecal scores of chicks were evaluated daily from the 4th day post-challenge till the end of the experiment by a method described by Du and Hu (2004), and then scored on a scale of 0–4, according to the droppings consistency and the presence of mucus/ blood in dropping (0 = normal droppings, 1 = normal to pasty, 2 = liquid, 3 = liquid and bloody, 4 = bloody droppings and abnormal fecal consistency).

4.2. Daily Oocysts shedding per gram feces and oocysts value:

Daily oocysts counting was done at 7th, 9th, 11^{th,} and 15th day post infection, by collecting the fecal droppings from the litter of each group. Fecal samples were examined for presence of oocysts according to Long and Joyner, (1976) using the hemocytometer method.

4.3. Body weight gain and relative weight gain:

All chickens of each group were weighted on the 1st day of age, then (weekly), at 7th, 14th, 21st days of age, then at the end of the experiment at the 30th day of age. Evaluation of growth parameters, which include weekly rate of food consumption, mean weight gain and growth rate for each bird was done according to Oyegoke *et al*. (2006).

4.4. Clinical signs

4.5. Mortality rate

4.6. Gross lesion score:

five chicks from each group at 7th day post infection were slaughtered and subjected to postmortem examination and then lesion scores were investigated, according to Johnson and Reid (1970). Gross examination of the gastrointestinal tract was done carefully. The intestine was divided into 4 regions, the upper one (duodenum and jejunum), the middle one (ileum), lower one (distal ileum and rectum) and two caeca. Gross lesions of GIT in any part of these regions were scored from 0 to 4 using lesion score key.

4.7. Histopathology:

Samples for histopathology were collected from duodenum, ileum, and caecum immediately after slaughter. Injection with 10% neutral buffered formalin solution in the lumen and then intestinal contents washed out. Samples then immersed in fresh neutral buffered formalin fixative for 48 hours. Samples were trimmed into longitudinal and transverse sections, then dehydrated into ethanol, and then embedded in paraffin wax according to the laboratory routine protocol. Rotary microtome (Leica RM2125) was used to cut samples and obtain 4µm sections from the paraffin blocks, and routinely stained with hematoxylin–eosin (H&E) stain. Optical images were cap-trued with an Olympus UC30 camera mounted on an Olympus BX41 optical microscope, and finally processed by Stream Basic analysis software (Pop *et al*., 2015).

5. Statistical analysis

Data obtained from the present study were tested for the significance of groups (G1,2,3…..12) treatments effect by ANOVA and GLM using the SAS Institute procedure (1990). Duncan's multiple range tests (1955) were used to determine differences among means when treatment effects were significant. Differences were significant when $p < 0.05$. Significant differences are denoted with different superscripts. Values

are presented as arithmetical means with standard deviation (Mean ±SD).

RUSULTS

1. Identification *Eimeria spp.* from collected samples:

In our study, the identification of *Eimeria* spp. were based on morphological characters of oocysts and sporocysts such as color, shape, form index, micropyle and its cap, and presence or absence of residual, and time of sporulation. Results of identification of collected samples revealed the presence of *E. mitis, E. maxima, E. acervuline, E. tenella,* and *E.* praecox. Table (1) shows morphological characterization of different *Eimeria* spp. oocysts.

2. Evaluation of the resistance of *Eimeria* spp. local isolates to anticoccidial drugs in Fayoumi chickens :

2.1. Anticoccidial sensitivity profile 1 (ASP1):

Results of anticoccidial sensitivity profile 1 (ASP1) are shown in table (2). Results indicated that there was full sensitivity of intestinal *Eimeria spp* local isolates to sulfaclozine, amprolium, and Diclazuril; and cecal *Eimeria spp.* isolates to sulfaclozine. There was partial resistance of intestinal *Eimeria spp* local isolates to toltrazuril and cecal *Eimeria spp* local isolates to amprolium, and Diclazuril. Complete resistance appeared in case of cecal *Eimeria spp* local isolates to toltrazuril.

2.2. Anticoccidial sensitivity profile 2 (ASP2):

Anticoccidial index (ACI) showed results that are similar to ASP1 except that amprolium showed full sensitivity to cecal *Eimeria spp.* local isolates, and toltrazuril showed partial resistance to intestinal and cecal *Eimeria spp.* local isolates. Results of anticoccidial index are summarized in table (3).

3. Evaluation of the efficacy of live attenuated vaccines and prebiotics for controlling Eimeria spp. infection in Fayoumi chickens:

3.1. Daily and total oocyst shedding per gram (OPG) feces:

Concerning to the mean of the total oocysts count per gram feces excreted in different groups during the experiment, the lowest oocysts count was detected in sulfaclozine group, followed by vaccine group, vaccine + prebiotic (agrimos) group, diclazuril group, prebiotic (agrimos) group, amprolium group, then toltrazuril group, as shown in table (4). The faecal matters of non-infected negative control group were free of *Eimeria* oocysts till the end of the experiment, while positive control group continued shedding a considerable high number of oocysts.

3.2. Mortality and survival rates:

Results of mortality and survival rates are shown in figure (1). There was no mortality in the negative control group, while high mortality (33.33%) appeared in the positive control group. The lowest percentage of mortality occurred in sulfaclozine and amprolium groups, followed by vaccine + prebiotic and diclazuril groups, then prebiotic group and toltrazuril group respectively.

3.3. Clinical signs

All infected chickens showed clinical symptoms of coccidiosis, pasty feces that tinged with blood were recorded in all infected groups. Clinical signs appeared at the 6^{th} and 7^{th} and disappeared at 9^{th} day post-infection. Clinical signs were more severe in the positive control group and continued for a longer time than other groups. negative control group showed no clinical symptoms.

3.4. Gross pathological lesions score.

Five chicks were slaughtered from each group and subjected to postmortem examination at day 22 of the experiment, and the gross pathological lesion score were recorded as shown in table (5).

3.5. Body weight gain.

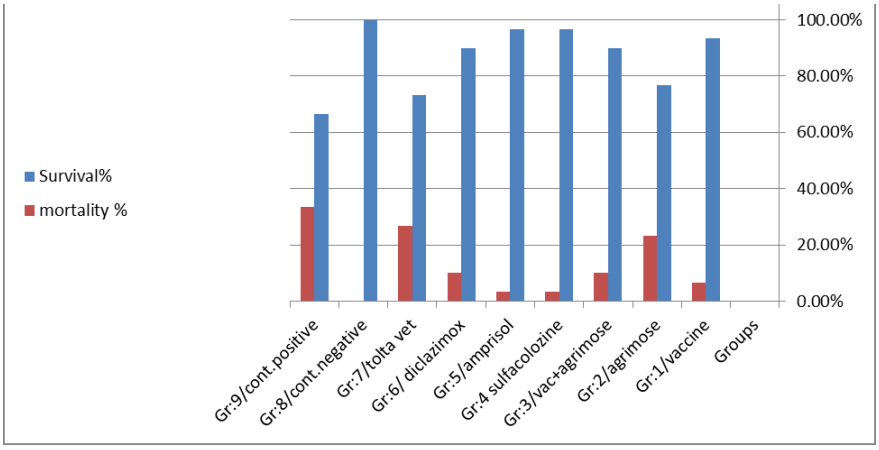
Concerning to body weight gain (grams/chick). At the 21st day after challenge, the highest body weight gain with no significant difference is negative control group, then amprolium group, vaccine + prebiotic (agrimos) group, sulfaclozin group, diclazuril group, toltrazuril group, vaccine group, prebiotic (agrimos) group in comparison with positive control group. The results of body weight gain are summarized in table (5).

3.6. Histopathological examination.

It was noticeable that the vaccine + prebiotic (agrimos) group and sulfaclozin group had lowest pathological lesions. Prebiotic (agrimos) group, amprolium group and diclazuril group had less severe pathological lesions. On the other hand, vaccine group, toltrazuril group, and positive control group showed more severe lesions. Histopathological lesions appeared as edema, sloughing and mononuclear cell infiltration, including eosinophil and lymphocytes, necrosis and desquamation of intestinal villi and hemorrhage. Results of histopathology are summarized in figure (2).

FIGURES:

Figure (1): shows mortality and survival rates in different groups.



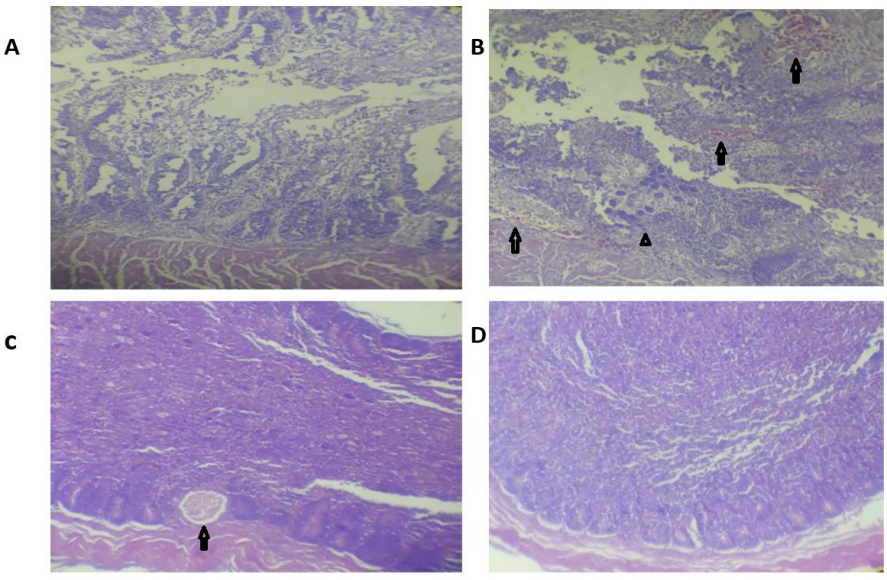


Figure (2): showing histopathological lesions in the positive control group. (A): sloughed epithelium of villi and numerous parasitized epithelial cells of crypts, (B): showing severe villous destruction with lymphocytic cells infiltration, hemorrhages (arrows) and cluster of coccidial gamonts (triangle), (C): congested blood vessel (arrow), and (D): showing caseous eroded surface over the intestinal mucosa and sexual stages of coccidia, (H&E x100).

TABLES:

Table (1): shows morphological characterization of different *Eimeria* spp. oocysts.

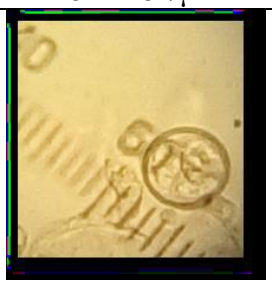
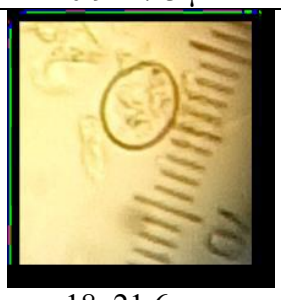
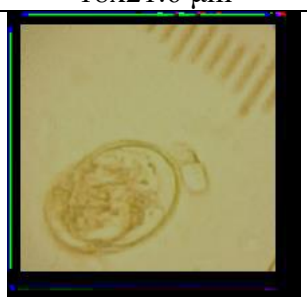
Eimeria spp.	**Morphological characters**
E. maxima	 28.4x18.7µm
E. mitis	 18x18 µm
E. acervulina	 20.9x17.3 µm
E. tenella	 18x21.6 µm
E. praecox	 21.5x16.2 µm

Table (2): shows results of anticoccidial sensitivity profile 1 (ASP1).

Equation	100% - (MLS of treated group /MLS of Eimeria challenge group x 100%)
Normal index	0 - 30 (complete resistance) 31 - 49 (partial resistance) ≥ 50 (full sensitivity)
Sulfaclozine treated group	Intestinal=100-(0.8/3.8 x100)=78.95 (full sensitivity) Cecal=100-(1/3.6 x100)=72.22 (full sensitivity)
Amprolium treated group	Intestinal=100-(1.6/3.8 x100)=57.895 (full sensitivity) Cecal=100-(2.20/3.6 x100)=38.9 (partial resistance)
Diclazuril treated group	Intestinal=100-(1.6/3.8 x100)=57.895 (full sensitivity) Cecal=100-(2.20/3.6 x100)=38.9 (partial resistance)
Toltrazuril treated group	Intestinal=100-(2/3.8 x100)=47.36 (partial resistance) Cecal=100-(2.8/3.6 x100)=22.2 (complete resistance)

Table 3: Shows results of anticoccidial index (ACI)ز

Equation	Survival + %weight gain relative to non-Eimeria challenge control) – MLS X10 + (mean number of oocysts /10^6) x0.4)
Normal index	ACI > 160 (full sensitive) ACI = 120 - 160 (partial resistance) ACI < 120 (complete resistant)
Sulfaclozine treated group	Intestinal : (96.6+87.015) - (8+0.52) = 175.095 (full sensitive) Cecal : (96.6+87.015) - (10+0.52) = 173.095 (full sensitive)
Amprolium treated group	Intestinal: (96.6+94.37) - (16+0.88) = 174.09 (full sensitive) Cecal: (96.6+94.37) - (22+0.88) = 168.09 (full sensitive)
Diclazuril treated group	Intestinal : (90+87.16) - (16+0.82) = 160.34 (full sensitive) Cecal : (90+87.16) - (22+0.82) = 154.34 (partial resist)
Toltrazuril treated group	Intestinal: (73.3+82.53) - (20+1.07)= 134.76 (partial resist) Cecal : (73.3+82.53) - (28+1.07)= 126.78 (partial resist)

Table 4: Shows the mean of total oocysts count per gram feces in different groups (p<.0001).

Groups	Mean Total oocyst count (M ± SD)
Group (1): Vaccine	$14.42x10^5 \pm 1.37^e$
Group (2): Prebiotic (agrimos)	$22.88x10^5 \pm 1.80^{bc}$
Group (3):Vaccine + Prebiotic	$17.33x10^5 \pm 0.93^{de}$
Group (4): Sulfacolozine	$13.02x10^5 \pm 1.70^e$
Group (5): Amprolium	$22.93x10^5 \pm 0.16^{bc}$
Group (6): diclazuril	$20.55x10^5 \pm 0.59^{cd}$
Group (7): Toltrazuril	$26.89x10^5 \pm 0.20^b$
Group (8): Negative control	0.00 ± 0.00^f
Group (9): Positive control	$57.42x10^5 \pm 4.01^a$

Table 5: Shows mean lesion score in different groups (p<.0001).

Groups	Intestinal lesion score	Cecal lesion score
Group (1): Vaccine	-	2.60 ± 0.40^{b}
Group (2): Prebiotic (agrimos)	2.80 ± 0.20^{b}	2.80 ± 0.20^{ab}
Group (3):Vaccine + Prebiotic	-	2.00 ± 0.32^{b}
Group (4): Sulfacolozine	0.80 ± 0.20^{d}	1.00 ± 0.00^{c}
Group (5): Amprolium	1.60 ± 0.24^{c}	2.20 ± 0.20^{b}
Group (6): diclazuril	1.60 ± 0.24^{c}	2.20 ± 0.37^{b}
Group (7): Toltrazuril	2.00 ± 0.32^{c}	2.80 ± 0.37^{ab}
Group (8): Negative control	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
Group (9): Positive control	3.80 ± 0.20^{a}	3.60 ± 0.24^{a}

Table 6: Shows mean body weight gain (grams/chick) in different treated groups (p<.0001).

Groups	Mean body weight gain (BWG)
Group (1): Vaccine	21.01 ± 1.29^{cde}
Group (2): Prebiotic (agrimos)	19.16 ± 1.09^{de}
Group (3):Vaccine + Prebiotic	24.66 ± 1.72^{abc}
Group (4): Sulfacolozine	24.26 ± 1.16^{abc}
Group (5): Amprolium	26.31 ± 1.63^{ab}
Group (6): diclazuril	24.00 ± 1.46^{abc}
Group (7): Toltrazuril	23.01 ± 1.38^{bcd}
Group (8): Negative control	27.88 ± 2.14^{a}
Group (9): Positive control	17.92 ± 1.24^{e}

DISCUSSION

coccidia infection is the most important intestinal parasitic disease of poultry worldwide. It causes high economic losses in poultry because of high mortality rates and high cost of medications. It is caused by one or more of nine species of *Eimeria* that affect poultry. The repeated and uncontrolled use of anticoccidials has developed drug resistance, which can be estimated variant criteria and indices (Chapman, 1998), so studying alternative control strategies against avian coccidiosis has become very essential.

Anticoccidial drug resistance is genetic and depends upon the oocysts number shedding from infected birds, the dose of oocysts infection, and the pre- exposure or re-infection immune status of chickens (Getachew *et al*., 2008). Although the use of anticoccidial agents over a long time may lead to the emergence of populations of coccidia resistant to used anticoccidials. So, it is essential to review or change periodically the anticoccidial drug used for prevention or treatment of coccidiosis (Chauhan *et al*., 2007). Mechanism of anticoccidial drug resistance may involve either appearance of different drug metabolism within the parasite and/or mutations at the binding site of the anticoccidial drugs (Taylor *et al*., 2007). It was indicated that resistance to amprolium and Sulfaquinoxaline has been developed in the field because of their intensive and uncontrolled use for over two decades (Anosa *et al*., 2011).

Results of the evaluation of the efficacy of anticoccidial drugs may be variable due to the use of different single parameters in this evaluation. However, based on the results of present research, both the ASPI and ASP2 gave similar results, and the dissimilarity is due to the narrower spectrum of classification of the ASP2. (Arabkhazaeli *et al*., 2013). In

our study, investigation of the resistance of the most common anticoccidial drugs against five field isolated *Eimeria* spp., two of the most common indices were used in the present study: ASP1 (McDogald *et al*., 1986), and ASP2 (Chapman, 1989). Anticoccidial sensitivity profile (ASP1) depends on reduction of lesion score (RILS), while anticoccidial sensitivity profile (ASP2) is based on anticoccidial index (ACI). ASP2 depends not only on lesion score, but the performance; weight gain, survival percentage; severity of parasite infection; oocyst production (Chapman, 1989). Drug resistance to anticoccidials under field conditions appeared in the form of a reduction in performance, decreased weight gain, and feed conversion rate (Stephen *et al*., 1997). So, depending only on intestinal lesion score, it may not be enough to provide adequate information to determine the anticoccidial drug sensitivity/resistance profile of *Eimeria* present in the field (Williams and Andrews, 2001).

Our results about Eimeria spp. resistance and sensitivity to sulfaclozine are in agreement with the findings of a study conducted by Harun-Rashid *et al*. (2016), who indicated that sulfaclozine was reported, tell now, effective in the prevention and treatment of coccidiosis. On the other hand, Amam *et al*. (2008) suggested that sodium sulfachloropyrazine monohydrate (sulfa drug) can only arrest development of the parasite, rather than destroying the parasite. It was stated that sulfadimidine is more effective than amprolium for treatment of coccidiosis, but overuse of anticoccidial drugs, such as sulfachloropyrazine has resulted in an increase in the emergence of drug resistance (Mathis *et al*., 1984; Abdisa and Kebede, 2016; Geng *et al*., 2021).

Our findings revealed that intestinal isolates showed full sensitivity to diclazuril by ASPI and SP2, but the cecal isolates showed partial resistance to diclazuril by ASPI andASP2. These results agree with Amer *et al*. (2007) who found that *Eimeria* spp. still sensitive to diclazuril (1%) liquid, and it is effective in preventing and/or controlling of the experimental and field coccidial infection. Efficacy of diclazuril as a feed additive in prevention and control of coccidiosis had been studied and proved to be highly effective (Chapman, 1989; Awaad *et al*., 2003; Meireles *et al*., 2003). It was investigated that the addition of diclazuril in feed or water was of the same effectiveness in eradication of experimental infection of *E. tenella, E. necatrix, E. acervulina, E. maxima* and *E. Brunetti* as shown by increasing body weight gain and survival rate, and reduction in faecal shedding, dropping score and lesion score (El-Banna *et al*., 2005). Also, the present study clarified that diclazuril had the capability of reducing the mortality rate and preventing the reduction of body weight gain caused by coccidiosis. These results agree with many studies which reported that administration of diclazuril in the drinking water was suitable for use in preventing and treating coccidial infection in chickens. This is indicated by decreased oocyst shedding, decreased lesion scores, and increased body weight in the treated chickens (El-Banna *et al*., 2005; El-Dakhly *et al*., 2006). The mode of action of diclazuril was studied by Varheven *et al. (*1989) and concluded that treatment with diclazuril mainly affects certain stages in the sexual development of *E. brunetti* and *E. maxima* resulting in complete eradication of the infection. It was stated that diclazuril can break down all intracellular developmental stages of asexual and sexual cycles of *E. tenella*, asexual later schizonts of *E. acervulina* and works against sexual and zygote of *E. maxima* and gametocytes of *E. brunetti* (Brander *et al*., 1991). Our result disagrees with this of Arabkhazaeli *et al*. (2013), who found that all isolated *Eimeria* spp. were resistant to the used anticoccidial drugs. All isolates were resistant to amprolium in combination with ethopabate and partial to complete resistance was reported to diclazuril. These may be due to difference of *Eimeria* isolates used.

Our study showed complete resistance to toltrazuril by ASPI and partial resistance by ASP2 for both intestinal and cecal isolates. Only one study was found about the resistance of *Eimeria* spp. to toltrazuril (Stephen *et al*., 1997), despite its regular use in the poultry field. So, it can be concluded that the resistance of Eimeria to toltrazuril does not appear to be a relevant factor in the poultry industry, in contrast to other anticoccidial drugs, such ionophors or quinolones in which resistances are more clarified (Vertommen *et al*., 1990). Adewole (2012) found that Toltrazuril is most effective anticoccidial drug for treatment of coccidiosis among common used drugs because it treated the largest percentage of infected birds. Early treatment with toltrazuril up to 36 hours after coccidial infection resulted in effective protection of chickens against coccidiosis. Variety of *in-vivo* factors can influence the efficacy of anticoccidial drugs, such as dilution by ingesta fluid, time of contact, absorption in GIT, and metabolism, and these factors must be considered (Alaa Eldin *et al.,* 2013).

The evaluation of the efficacy of anticoccidial agents depends mainly on the anticoccidial indices, such as survival rate, lesion score, oocyst count and relative weight gain (Shah *et al*., 2009; Ma *et al*., 2011). There are new approaches that can be used for prevention and control of coccidiosis. These approaches include using natural products, prebiotics, live attenuated vaccines, standard management practices, and improvement of chicken immune status (Allen and Fetter, 2002; Dalloul and Lillehoj, 2006; Titilincu *et al*., 2008; Abbas, 2012).

Many vaccines are used worldwide to control Eimeria infection in chickens. It was reported that specific species of Eimeria can express immunological variation, and preparation of live vaccines from these *Eimeria* spp. can affect efficacy of vaccine in terms of stimulation of cellular and humoral responses (Conway and McKenzie 1991; Fitz-Coy, 1992; Lee, 1993; Conway *et al*., 1999; Gautam *et al*., 2005). Lymphoid organs including bursa of fabricius, spleen, thymus and cecal tonsils are responsible for immune response against pathogens that can invade the intestine (Lillehoj and Lillehoj, 2000). Antibodies such as IgA, IgG and IgM begin to be produced soon after natural Eimeria infection (Lillehoj and Lillehoj, 2000) or vaccination (Ayaz *et al*., 2008) with an effective protection of intestinal mucosa and significant reduction in severity of clinical symptoms and mortality rates.

Regarding to the mean total oocyst count, all of vaccine-treated groups, were significantly lower than the control positive group, but the vaccine-only-treated group was the lowest one then vaccine + prebiotic, then the prebiotic group. The oocyst count was significantly different. Increased fecal oocyst count may have a strong relationship with the coccidiosis severity. This may be due to the great variation in the oocyst shedding at different time intervals of the infection (Reid (1975). The present data indicate that vaccine (Fortegra) produced protection in chickens infected with cecal Eimeria isolates. This protection was indicated by decreased rate of mortality in vaccinated groups; increased body weight, improved weight gain, decrease in gross lesion score, decreased dropping score, and decreased oocyst shedding. These findings agree with many studies (Williams and Gobbi, 2002; Afshin Zakeri, 2011; Lee *et al*., 2013). Lesion score and body weight gain in vaccine + prebiotic group was higher than vaccine only, this may be because of prebiotic (agrimos). It was stated that the protective effect of manan-oligo-saccharide could be due to increase in length of villi and improved integrity of the GIT (Loddi *et al*., 2002). Decreased gross lesion scores and oocyst count by administration of feed additives may be related to reduced pH and increased beneficial microflora. The present results disagree with a study conducted on infected chicks with several species of Eimeria. It was shown that by addition of prebiotic to feed, only gross lesions caused by *E. acervulina* were significantly reduced in the intestines of

infected chicks, but prebiotic produced no protection against infections of *E. tenella* and *E. maxima* (Elmusharaf *et al*., 2007). Prebiotics produce protection, which may be a result of inhibition of asexual schizonts development after stimulation of cellular immune responses (Elmusharaf *et al*., 2006). Many studies tried to explain the beneficial effect of prebiotics in the control of poultry coccidiosis. In fact, prebiotics may simultaneously stimulate the cellular immune system, which protects birds from intestinal parasitism, and produce local secretory antibodies during natural infection with *Eimeria spp.* (Gomez-Verduzco *et al*., 2009). It was reported that the combination of prebiotics and vaccination increases the protection of chickens against Eimeria infection, with increased stimulation of immune response and increased performance (Nollet *et al*., 2007). The use of prebiotics increases the cellular lymphocytes proportion and humoral IgA titer in the ileum (Swanson *et al*., 2002; Li *et al*., 2007). Prebiotics increase the length of villi and facilitate their regeneration, which may play a role in the protection of the intestinal mucosa in the case of inflammatory reactions caused by intestinal pathogens or toxins (Gao *et al*., 2008). Prebiotics can act as competitor inhibitors with sporozoites for binding sites on epithelial cells lining the intestine, and so can reduce the adhesion and the subsequent proliferation of *Eimeria* spp. (Shoaf *et al*., 2006). It was suggested that the lower effect of prebiotics (agrimos) group only than other treated groups may be due to the type and source of prebiotics, the time and dose of their use in the feed, count of oocyst, and *Eimeria spp.* can affect the indicators measured in different experiments (Behnamifar, *et al*., 2019). Our results showed that the use of prebiotic(agrimos) only was partially effective, and its protection was not enough to be used as a substitute for anticoccidial drugs. This agrees with Taherpour *et al*., (2012) who showed that prebiotics can increase the resistance of chickens and partially protect against coccidiosis. So, prebiotics in combination with some supplements, such as salinomycin, can partially finish the adverse effects of coccidiosis.

Our results of histopathology of the infected untreated positive control group go parallel with the results of Zulpo *et al*. (2007) and Amer *et al*. (2010). Inflammatory reactions in the infected untreated group may be due to the invasion of intracellular developmental stages of Eimeria protozoan in the gut. It was reported that several pro-inflammatory cells, T-helper cell type 1, cytokines and chemokines were increased in the chicken gut following infections with *E. tenella*, *E. acervulina*, and *E. maxima* (Hong *et al*., 2006). These inflammatory mediators have been suggested to have partial responsibility for intestinal damage during coccidiosis (Hong et al., 2006b). It was reported that oxidative stress induced by the Eimeria infection could stimulate development of coccidial intestinal lesions (Allen, 1997).

Present histopathological findings of toltrazuril were disagree with many studies (Lakkundi *et al*., 2002; Ashraf *et al* 2009, Ashraf 2011; Hagag *et al* 2020). While histopathological findings of diclazuril agree with El-Banna *et al*. (2005), Amer *et al*. (2007) and Assis *et al.* (2010). Histopathological findings of sulfaclozin agree with Hagag *et al*. (2020). Histopathological findings of amprolium agree with Lakkundi *et al*. (2002).

Our histopathological findings show that the vaccine + prebiotic (agrimos) group had the lowest pathological lesions, and the prebiotic (agrimos) group had less pathological lesions. These findings agree with Al-Baadani *et al*. (2016). Similar studies revealed that the use of probiotics and prebiotics as feed additives led to improvement of the morphometric histological status and absorption surface, and reduction of pathogenic bacteria (Awad *et al*., 2009).

CONCLUSION

It was concluded that using morphological methods for identification of Eimeria spp. in Fayoumi chickens, five *Eimeria* spp. could be isolated; *E. mitis*, *E. maxima*, *E. acervuline*, *E. tenella*, and *E.* praecox. The current study proved the presence of resistance of isolated *Eimeria* spp. to toltrazuril and good sensitivity to sulfaclozine and amprolium in fayoumi chickens. This study showed that living attenuated vaccine (Fortegra) is effective for control of coccidial infestation, and better results will be obtained in case of addition of prebiotic (Agrimos) to the vaccine. The use of prebiotic (Agrimos) in control of coccidiosis is not effective.

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تقييم مقاومة المعزولات المحلية لأنواع الايميريا لمضادات الكوكسيديا وفاعلية اللقاح المضعف والمعززات الحيوية فى السيطرة على عدوى الايميريا فى الدجاج الفيومى

أحمد خلف حسن ، ورده ممدوح محمود حسن ، مصطفى عبد المطلب شحاته ، محسن إبراهيم عرفه

E-mail: a.hassan@aun.edu.eg Assiut University web-site: www.aun.edu.eg

يعتبر مرض الكوكسيديا فى الدواجن واحدا من اهم الامراض الداجنه اقتصاديا حيث انه مسئول عن خسائر اقتصاديه كبيرة في الصناعه الداجنه عالميا .ويحدث هذا المرض نتيجة العدوى بنوع او أكثر من طفيل الايمريا .. وتكمن مشكلة مرض الكوكسيديا فى الوقت الحالى فى ان تكرار استدخدام الأدوية الوقائية أو العلاجية مع مرور الوقت تصبح غير مجدية وتحدث العدوى بسبب مقاومة طفيل الايمريا لتلك العلاجات.

ولذلك هدف هذا العمل الى دراسة :

أولاً: تقييم مقاومة المعزولات المحلية لأنواع الأيميريا لمضادات الكوكسيديا

ثانيا: تقييم فاعلية اللقاح المضعف والمعززات الحيوية فى السيطرة علي عدوى الايميريا في الدجاج الفيومي.

أجريت هذه الدراسة بمحطة بحوث الانتاج الحيوانى بملوى. وقد استخدمت فى هذه الدراسه خمسة انواع من الايمريا هي ايمريا تنلا ,ايمريا ميتس, ايمريا ماكسيما وايمريا اسيرفيولينا وايمريا برياكوكس تم تجميعها من الدجاج المصاب طبيعيا بعدوى الايميريا. وتم عمل عدوى تجريبية فى عدد ٢٧٠ كتكوت تم تقسيمها الى تسع مجموعات عند عمر يوما٠

وقد تم تقييم التجربة من خلال مقارنة تأثير المعاملات المختلفة على الزيادة في وزن جسم الطائر , بالإضافة إلى متوسط أعداد أكياس الكوكسيديا بعد العلاج والإعراض المرضية والهستوباثولوجيه.

ولتقييم مقاومة الادوية لانواع الكوكسيديا تم استخدام نوعين من القياسات:

١- ASP1 ٢ - ASP2

وأوضحت النتائج :

- حساسية المعزولات المحلية للانواع الايميرية المختلفة للسلفاكلوزين والامبروليم والدايكلازوريل .
- مقاومة المعزولات المحلية للانواع الايميرية المختلفة للتولترازوريل.
- فاعلية اللقاح المضعف سواء استخدم منفردا او مع خليط من المعززات الحيويه في السيطره علي العدوى الايميريه في الدجاج الفيومي.
- ضعف فاعلية المعزز الحيوي عند استخدامه منفردا في السيطره علي العدوى الايميريه في الدجاج الفيومي.