Comparison between prophylactic drugs and vaccination against coocidiosis in chicken

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Thank you

Dedicated
To

My parents
My wife, daughters & son
My brothers & sisters
My family
My professors
All my friends

With

My special thanks, and
gratefulness

Mostafa

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LIST OF ABBREVIATIONS

BW gain	Body weight gain
E. spp.	*Eimeria* species
FCR	Feed conversion rate
IL	Interleukin
$K_2Cr_2O_2$	Potassium dichromate
MBW	Mean body weights
MBW gain	Mean body weights gain
NO	Nitric oxide
OPGC	Oocyst per gram counts
PI	Post infection

INTRODUCTION

The poultry industry plays an effective role in the human generating revenues, and is in general vital in the national economy comprising source of animal protein either meat or egg **(Nnadi and George, 2010)**. Chicken coccidiosis comprising the most important protozoan related diseases worldwide **(McDougald and Reid, 1997; McDouglad, 2003)**. It is considered as one of the most economically important and common diseases in spite of the advancement of chemotherapy, biosecurity, nutrition, or genetics **(Mcdougald and Raid, 1991)**.

Avian coccidiosis possess economically important changes resulted in delayed growth, decreased food conversion and depigmentation. Also, it can produce changes in the metabolism, tissue constituents and dietary requirement which adversely affect poultry production **(Allen, 1986)**. It constitutes a major problem can seriously threaten the poultry industry. It is responsible for pronounced economic losses **(McDougald and Reid, 1997; McDouglad, 2003)**. This parasitic disease is evaluated to waste the poultry industry by 3.2 billion USD annually **(Dalloul and Lillehoj, 2006; De Gussem, 2007)**. It is associated with weight loss, lowered feed intake, delayed sexual maturity and decrease in the egg production.

Several species of Eimeria cause coccidiosis in chickens, with the most prevalent E. tenella, E. acervulina and E. maxima. All E. spp. parasitize the intestine, generated pathological changes varying from the local mucosal destruction to the systemic effects such as hemorrhage, shock, and death **(Vermeulen et al., 2001)**. Previously, poultry industry personnel have controlled coccidiosis through usage of the anticoccidial feed additives.

It has been applied over 50 years in prevention or treatment of coccidiosis in poultry **(Chapman, 1997)**. These anticoccidial agents influence essential biochemical pathways of the parasites through affecting an important co-factor of such pathway **(Greif et al., 2001)**. Vaccination program through using a live oocyst is currently a realistic compensated to anticoccidial drugs for prevention of coccidiosis in broilers **(Chapman et al., 2002)**. Over the past decade, the usage of coccidial vaccines has increased due mostly to side effects related to ionophores.

In broiler, the prevention and control of protozoal coccidiosis has been targeted several years ago through the use of two main tools: anticoccidial agents through the feed **(Chapman, 2000)** and live vaccines either attenuated or non-attenuated live oocysts **(Kitandu and Juranova, 2006)**. Bird's vaccination seems to be safer and promising means to control avian coccidiosis **(Martin et al., 1997)**.

Aim of the work:

The present study was constructed to compare the relative effectiveness of two disease control drugs; amprolium and diclazuril) and vaccination (Coccivac D, live oocyst vaccine) in an experimental research facility. Clinical signs, dropping scores, mean lesion scores, mortality %, oocyst counts and production indices were parameters for evaluation of the performance and the efficacy level of prevention of coccidiosis. Moreover, pathological changes were be detected.

REVIEW OF LITERATURE

1- General description on poultry coccidiosis:

Graat et al., (1996) stated that coccidiosis is considered one of the common and major poultry disease problems in spite of advances in prevention and control through chemotherapy, management and nutrition.

Williams, (1996) showed that avian coccdia is protozoan belonging to genus *Eimeria* species; it multiply in the intestinal tract resulted in disturbance in the feeding and digestive processes with increased susceptibility to other disease agents.

Allen et al., (1997) noted that coccidiosis is constituted as the parasitic disease associate with the greatest economic impact on poultry production. Annually, with approximately 80% of these costs attributed to decrease performance in the presence of drug treatment strategies.

Williams, (1998) defined that avian coccidiosis is a common parasitic disease of broiler caused by single protozoan parasite of the genus *Eimeria* that colonize the intestinal tract. Coccidiosis induced a substantial economic cost to the poultry industry that is calculated on more than $800 USD million in annual losses. Losses are attributed to feed medication used in the control and treatment, mortality, malabsorption of the nutrients, inefficient feed utilization, and impaired growth rate.

Julie, (1999) mentioned that coccidiosis is a disease of poultry caused by a protozoan lives and multiplies in the intestinal tract and causes tissue damage.

This damage can interfere with the food digestion and nutrient absorption, as well as causing dehydration and blood loss. The tissue damage makes bird more prone to bacterial infections, like clostridium and salmonella. Diseases that suppress the bird's immune system may act with coccidiosis to produce a more severe problem. Such as, Marek's disease may interfere with the development of coccidiosis immunity and Infectious Bursal Disease may exacerbate a coccidia infection.

Xie et al., (2001) displayed that coccidiosis is an economically important poultry disease caused by many species of microscopic eukaryotic protozoan parasites of the genus *Eimeria* which related to the phylum Apicomplexa. Coccidiosis is more linked to intensive animal production systems. In modern poultry rearing, high stocking densities of susceptible young birds provide an ideal environment for development and reproduction of coccidia. Coccidiosis is the most commonly reported poultry disease all over the world.

Allen and fetterer, **(2002)** stated that coccidiosis is an old well-recognized parasitic disease. This disease is more prevalent throughout the country and has a significant economic impact on poultry.

McDougald, (2003) reported that coccidiosis is a disease of almost universal importance in poultry production. The disease may strike any type of poultry in any type of facility. The parasite multiplies in the intestine caused tissue damage, resulting in diminished feed intake and nutrient absorption, reduced body weight gain, dehydration, hemorrhage and increased susceptibility to bacterial diseases.

Williams, (2005) recorded that coccidiosis is a disease of major economic importance in poultry industry. It is a widespread disease of growing chickens that can seriously hinder and delay the development of poultry production **(Conway and Mckenzie, 2007)**. The protozoan parasite of the genus *Eimeria* inhabitant the intestine associated with growth problems with increased susceptibility to other disease pathogens **(McDougald and Fitz-Coy, 2008)**.

Conway and McKenzie, (2007) observed that coccidia of the genus *Eimeria* are predominately host-specific, each species occurs in a single host species or a group of closely related hosts. Infection by coccidia is caused by sufficient numbers to produce clinical manifestations of disease that is termed coccidiosis. Differential identification of each species is dependent upon the following characteristics; zone of intestine parasitized, gross appearance of the lesion, oocyst morphology minimum sporulation time, minimum prepatent time schizont size, location of parasite in the host intestinal epithelium and cross immunization tests.

Pangasa et al., (2007) defined coccidiosis is as one of the serious poultry diseases that infect the intestines lining. It is a complex disease caused by different species of *Emiria* parasite. The damaged tissue caused by coccidia results in lowered feed intake, interference with normal digestion and nutrient absorption, dehydration and blood loss.

Kahn, (2008) stated that a nine species of *Eimeria* have been identified as causative agents of poultry coccidiosis; only seven of them have been detected as to be pathogenic.

Jadhav et al., (2011) displayed that avian coccidiosis is a worldwide problem in poultry induced a huge economic loss to poultry industry attributed to the occurrence of different *Eimeria* species combinations and the intensity of infection.

2-Etiology of coccidiosis in poultry:

McDougald, (1998) stated that coccidiosis is disease affect poultry and is caused by a protozoan parasite known as *Eimeria*. A numbers of *Eimeria* species have been recorded from poultry which are affecting a particular part of the intestinal tract as shown in **Table, (1)**.

Thebo et al., (1999) mentioned that there are a nine *Eimeria* species have been identified of which *Eimeria brunette*, *E. maxima*, *E. necatrix*, and *E. tenella* are pathogenic, while E. acervulina, *E. mitis*, *E. praecox*, *E. hagani*, and *E. mivati* are considered as non-pathogenic.

Vermeulen et al., (**2001)** showed that several species of *Eimeria* cause coccidiosis in chickens, with the most prevalent *E. tenella, E. acervulina* and *E. maxima*. All *Eimeria* spp. parasitize the intestinal lining, causing remarkable pathological changes varying from local destruction of the mucosa to systemic deteriorations such as blood loss, shock, and death.

Allen and Fetterer, (2002) exhibited that there are seven species of *Eimeria* that parasitize chickens (*Gallus gallus*). These species are *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox, E. tenella* and they occur throughout the world wherever domestic fowls were recorded.

McDougald, (2003) reported that *Eimeria* species is the causative agent of coccidiosis in poultry. There were criteria that are useful in the identification of *Eimeria* species as following 1. Location of the lesion in the intestine. 2. Macroscopic appearance of the lesions. 3. Oocyst size, shape, and colour. 4. Size of schizonts and merozoites. 5. Minimum prepatent period in experimental infection. 6. Location of the parasite in the tissues (type of cell parasitized). 7. Immunogenicity against reference strain.8. Stage of the life cycle that produces most tissue damage. 9. Molecular and biological approach.

Conway and Mckenzie, (2007) mentioned that coccidia consist of a wide variety of single cell parasitic animals in the sub-kingdom Protozoa, phylum Apicomplexa. It was recognized as nine different species; of these, seven *Eimeria* occur in chicken-namely, *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella*. Each species attacks a different intestinal part or ceca and causes a separate disease exhibiting a characteristic degree of pathogenicity.

Chere, (2013) showed that six of the 7 pathogenic *Eimeria* species known to parasitize chickens were occurred as single or multiple infections in broiler farms. *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix* and *E. tenella*. *Eimeria tenella* was the predominant species. The occurrence of subclinical form of infection was higher, and mostly associated with various *Eimeria* species.

Chapman, (2014) recorded that coccidia infection caused by *Eimeria* species that differ according to pathogenicity. Whereas, *E. mitis and E. acervulina* is less pathogenic species, but *E. tenella and E. necatrix* is considered a highly pathogenic.

Table 1: Species of *Eimeria* with their predilection site in the host according to **McDougald, (1998)**.

Site of lesions	species
Caecum	*E. tenella*
Duodenal loop	*E. acervulina*
Mid gut	*E. necatrix*
Mid gut	*E. maxima*
Anterior gut	*E. hagani*
Duodenal loop to rectum and caecum	*E. mivati*
Anterior gut	*E. praecox*
Anterior gut	*E. mitis*
Lower intestine	*E. brunette*

3- Life cycle of Eimeria species:

Soulsby, (1982) showed that the first generation of schizont measured 54 µm diameter and may contain up to 900 first generation of meroziotes. The mature schizont ruptures into the lumen of the crypts of the caecal glands 3 days post infection (PI) and the merozoites penetrate other epithelial cells to form young second generation of schizonts. Colonies of the second generation of schizonts mature by day 4 post infection (PI) and release about 300 second generation of merozoites into the lumen of cecum. When large numbers of second generation of schizonts are involved, a massive hemorrhage with blood escape into the caecal lumen may be evident at about day 4 PI.

Lillehoj and Trout, (1993) expressed that the typical life cycle of *Eimeria* involved three major phases namely sporogony, merogony (schizogony) and gametogony.

Shirley, (1995) reported that although the general life cycle is the same for all *Eimeria*, host specificity, site of development, patent and prepatent periods and pathogenicity vary between species.

Williams, (1995) discussed that *Eimeria* has a short life cycle, which, depending on the species, takes 4 to 6 days. The life cycle is direct without the involvement of an intermediate host. The infective stage of the organism is a thick double walled oocyst which on release from the host can persist in the environment for a long time. A typical *Eimeria* life cycle has 3 stages: sporogony, schizogony or merogony, and gametogony. Sporogony is the process by which the oocysts contained zygote in the environment undergoes a reduction division to form four haploid sporoblasts. Sporoblasts develop to form sporocysts each with a distinct cell wall. Each sporoblast divides mitotically to produce two sporozoites. Hence the *Eimerian* oocyst at this stage has eight sporozoites.

Allen and Fetterer, (2002) noted that four sporocysts each containing two sporozoites are formed within each oocyst during sporogony occurred outside the host under suitable environmental conditions (warmth, oxygen and moisture). Sporulated oocysts, when ingested by susceptible hosts, initiate the infective cycle.

Jeurissen and Veldman, (2002) showed that infection with coccidiosis follows the ingestion of viable oocysts, which are contaminants of food, dust,

and water. *Eimeria* exhibit a complex life cycle comprising stages both inside and outside of the host. During the in-host stage, there are both intracellular and extracellular stages and both asexual and sexual reproduction. This complexity provides the immune system with only three moments to inhibit *Eimeria* development. After the oocysts are swallowed, they are subjected to the action of the digestive enzymes in the upper intestine and the grinding process in the gizzard, which lead to the liberation of sporozoites (excystation). Following the liberation, the sporozoites actively penetrate the epithelium of the intestine, and are then transported in macrophages through the lamina propria of the villi to reach the epithelium at the depth of the intestinal glands, where further developments occur. Most Eimeria species have a characteristic site of invasion, and in chickens, these locations are used as diagnostic features. Following the penetration of the epithelial cells there is a period of growth during which the parasites becomes rounded, and is now termed trophozoites.

McDougald, (2003) mentioned that *Eimeria* exhibit a complex life cycle, comprising both endogenous and exogenous stages. The endogenous development process has asexual and sexual reproduction occurred inside gut epithelial cells of the bird, while exogenous development involves maturation of the oocysts outside bird by sporulation. After ingestion excystation, the released sporozoites invade the intestinal epithelium and round up to form a trophozoite followed by nuclear division to form an immature meront (schizont) by which the merogony stage commences. Different numbers of merozoites are being produced asexually through multiple fission process by each meront. *E. tenella* has 2-3 generations of merogon.

Conway and McKenzie, (2007) recorded that second generation of merozoites penetrate new epithelial cells and initiate either third generation of

schizonts or the gametogonous cycle with the majority undertaking gametogony cycle. Gametogony starts when merozoites invade cells and develop into either macrogamonts or microgamonts. The former gives rise to a single macrogamete whereas the latter undergoes multiple divisions resulting in the formation of numerous flagellated microgametes. Fertilization occurs when the microgamete invades cells containing macrogamete and a wall forms as oocysts mature.

4-Epidemiology:

Hofstad, (1984) recorded that onset of the avian coccidiosis depends on the age of the bird at the time of the first infection and number of passages of the infection (for one passage to be completed it is required 10 days), as well as on capability of the bird to develop proper specific immune response.

Abu Elezz, (1994) stated that the cecal coccidiosis caused by *E. tenella* which is the most prevalent species in Balady chicks in Egypt. However, **Haug et al., (2008)** recoded *E. tenella and E. maxima* were the most prevalent species associated with medium-sized and large oocyst, respectively in broiler chickens in Norway.

Calnek, (1997) mentioned that distribution and prevalence of coccidiosis is depending on several factors: high animal density cramped one small space, high air temperature, high relative humidity, different categories of birds at the same place especially of different ages, feed change, quality of feed, as well as other factors that compromise resistance to the disease and general health status of the birds. It is impossible under farming conditions to produce a coccidia free environment **(Jordan et al., 2002)**.

Mc Dougald et al., (1997) found several species of *Eimeria* oocysts from 15th and before 21th days old in the flocks. The differences of age susceptibility between native breed (Balady) and normal broiler might related to genetic factors.

Razmi and Kalideri, (2000) confirmed that the prevalence of coccidiosis significantly increased with an increasing size of flocks. However, the prevalence was remarkable lower in the large-scale broiler farms with large flocks.

Ahmed et al., (2003) recorded that the presence of *E. acervulina, E. maxima and E. mitis* species constitutes 43.9% in Egypt.

Ashenafi et al., (2004) confirmed that the incidence of coccidiosis is varied according to different selected climatic zones; there were a significant difference in coccidiosis prevalence in chickens.

Shirley et al., (2005) recorded that *Eimeria* parasite is transmitted via a resistant oocyst and infection occurs in a susceptible chicken through ingestion of the sporulated oocyst from the environment.

Kiani et al., (2007); Taylor et al., (2007) noted that the oocysts wall offered an effective protective barrier against the extremes of environmental conditions and mechanical disruption, as such *Eimeria* oocysts can be mechanically spread to poultry houses by different routes such as dust, boots, cloths, wheels, contaminated equipment and personnel who move between pens, houses or farms.

Haug et al., (2008) found the incidence of *E. acervulina and E. maxima* was 100% and 27.5%, respectively in broiler chickens in Norway.

Nematollahi et al., (2009) reported that the prevalence of Eimeria species vary by flock size. Since, the highest prevalence rate recorded in the small-scale flocks and the lowest in the large-scale flocks related to the managemental practices in the small- and large-scale broiler farms.

Ahmed et al., (2012) recorded that different age susceptibility among Egyptian native breed of different *Eimeria* species, *E. acervulina* and *E. tenella* occurred in 4th week and in older ages. In the contrary, *E. necatrix*, *E. maxima* and *E. mitis* infections weren't begin before 42 days of age.

5- Factors influencing the occurrence of coccidiosis:

Urquhart et al., (1987) reported that poultry coccidia have high capacity to reproduce within the host resulting in a rapid increase to success and the subsequent high level of parasite within the susceptible host and subsequently high level of contamination of the environment.

Jordan et al., (2002) found that the occurrence of poultry coccidiosis is dependent on both the species of *Eimeria* and the size of the infecting dose of oocysts. The number of oocysts in the litter rises rapidly that associated with the short prepatent period of the parasite and its high biotic potential.

Fanatico, (2006) defined that coccidiosis is usually a disease of young birds that can be infected at any time, if never exposed before. Coccidia

populations take a time to build up dangerous levels; therefore, outbreaks usually occur when birds are between 3 and 8 weeks of age.

Adhikari et al., (2008) showed that management of poultry houses plays a momentous function in the spread of coccidiosis because coccidial oocysts are omnipresent and are rapidly spread in the poultry house environment. Further, owing to their high reproduction potential, it is very complex to keep chickens coccidia free, especially under current intensive rearing conditions. Prevalence of coccidiosis varied by management and did not vary by flock size **(Hadipour et al., 2011)**.

Al-Quraishy et al., (2009) displayed that the bad management including wet litter that encourages oocyst sporulation, contaminated drinkers and feeders, bad ventilation, and high stocking density can initiate the clinical signs.

6- Clinical signs of coccidiosis:

Jordan, (1990) mentioned that the first and the most frequent symptom is yellow diarrhea, because of the blood in feces, feces arered or resemble the color of chocolate. Clinical symptoms appeared at the time when the second generation of shizonts starts rapidly to replicate, grow, mature and release the second generation of merozoits that induced inflammation of the sub epithelial mucus, desquamation of the lining epithelial and rupture of blood vessels in the caecum wall. Consequence, bloody diarrhea occurred.

Calnek, (1997) reported that the feathers around the cloacae are covered with bloody deposits. Feces are stained with blood. Birds that survive first few

days of the infection, can survive the next 10 to 15 days. During that time, birds are thirsty and rapidly lose weight.

Kidd et al., (2003) found that coccidial infection adversely affect broiler body weight; since it exhibited poor growth performance.

McDougald, (2003) mentioned that mortality were highest rate recorded between the fourth and sixth day, death sometimes occurring unexpectedly due to excessive blood loss.

Simon M., (2005) showed that coccidiosis is generally acute in onset and is characterized by depression, ruffled plumage and diarrhea. Birds infected with *E. tenella* showed paleness comb and wattles and bloody stained droppings.

Williams, (2005) mentioned that coccidiosis led to decreased body gain and feed conversion ratio deterioration, as well as increased incidence of diarrhea and intestinal hemorrhage which have economically significant impact on the poultry industry. As well, there were other pathophysiological effects associated with poor feed efficiency, reduced water intake, increased intestinal passage time, decreased digesta viscosity, intestinal malabsorption, villus atrophy, intestinal leakage of plasma proteins and increased intestinal activity.

Taylor et al., (2007) showed that the first sign of coccidiosis becomes noticeable at about 3 days after infection on flock basis. Refusal to feed and drink is considered the first sign was detected. Chickens showed droopiness, huddle to keep warm and passed out bloody diarrhea. Cecal blood loss with characteristic odour was noticed shortly before mortality begins.

McDougald and Fitz-Coy, (2008) confirmed that the protozoan parasite of the genus *Eimeria* multiplies in the intestinal tract and causes tissue damage, resulting in the interruption of feeding, digestive processes, nutrient absorption, dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease pathogens.

Nematollahi et al., (2009) mentioned that chickens suffering from coccidiosis are quickly become less productive and poor performers. Laying hens exhibited a reduction in rate of egg production.

Amer et al., (2010) recorded that coccidiosis induced intestinal lesions and loss of pigmentation which become apparent during the latter stages of infection.

7- Effect of coccidiosis on body weight gain:

Kettunen et al., (2001) reported that *Eimeria*-infected chickens showed decrease in the feed intake than the non-infected chickens, which adversely impact weight gains. This decrease in food intake and malabsorption might also be correlated with poor immune status.

Vermeulen et al., (2001) found that coccidia infection leads to huge economic losses resulting from nutrients malabsorption, which causes decreased MBW gain, poorer FCR, and possibly increased mortality.

Allen and Feterrer, (2002) mentioned that chickens coccidiosis induced decreased weight gain. Conversely, feed conversion also occurred because

intestine epithelial cells are damaged by infection and impairing nutrient digestion with malabsorption.

Gautam et al., (2005) recorded that infection of birds in the experimental subgroups with two strains of *E. tenella* resulted in a significant reduction in performance parameters including FCR, final BW gain, and mortality rate. The reduction in growth take place due to the existence of cecal lesions caused by *E. tenella* and the subsequent malabsorption of nutrients, anorexia, and listlessness of birds and the reduction in performance parameters is most characteristic signs by Matrouh isolate. Mortality due to *E. tenella* is intensively influenced by the pathogenicity and virulence of each strain.

Lobago et al., (2005) mentioned that coccidiosis led to weight loss, lower in the feed conversion rate, delayed sexual maturity and decrease of egg production.

Williams, (2005) detected that coccidia infection induced decreased body gain and feed conversion ratio, as well as increased incidence of bloody diarrhea and intestinal hemorrhage.

Awais et al., (2012) showed that chicken with coccidiosis recorded remarkable signs characterized by dysentery, enteritis, emaciation, drooping wings, poor growth and lower production.

Zhang et al., (2013) recorded that coccidiosis has major economic impacts on poultry with lesser body performance and decreasing productivity.

El-Morsy et al., (2016) showed that coccidia infected birds had severely clinical signs, mortality rate, lesion score, oocyst output, weight gains, FCR values, body weights and sporulation percent in comparison with treated birds. Clinical symptoms of coccdia infection revealed ruffled feather, depression, huddle together, decrease appetite, emaciated breast muscle, knife edged keel bone and bloody diarrhea. Severely enlarged two cecae with thickened mucosa, bloody cecal core and blooning were the most prominent lesions post *E. tsunodai* infection.

8- Necropsy findings:

Hein, (1971) found that *E. acervulina* exhibited presence of gametocyte with the remarkable inflammatory cells in duodenum. Moreover, *E. necatrix* showed its characteristic coagulative necrosis and focal hemorrhagic areas and deeply embedded gametocyte in tunica musculosa and serosa.

Levine, (1985) noted that *E. tenella* exhibited considerable numbers of oocyst in cecal lumen beside severe hemorrhage and complete epithelial desquamation and muscular edema.

Marquardt et al., (2000) recorded that the thickening in gut wall due to coccdiosis is indicating retention of fluid (edema). Hemorrhage with blood loss or merely retention of an excessive amount of blood in the tissue (hyperemia) was observed. Also infiltration with various body reactions and the development of immune responses was detected.

Vermeulen et al., (2001) showed that several species (spp.) of *Eimeria* (E.) caused coccidiosis in chickens, with the most prevalent *E. tenella*, *E.*

acervulina and E. maxima. All E. spp. led to systemic effects such as hemorrhage, blood loss, shock, and death.

Fanatico, (2006) noted that coccidiosis caused intestinal thickening which seemed as sausage. There may be light colored spots on the surface of the gut and inside the gut hemorrhages and streaks. The type and locations of lesions in the gut differ according to *Eimeria* species. *Eimeria acervulina* affects the upper parts of the small intestines which seen as small red spots and white bands on it; *E. maxima* affect the entire small intestine where the intestine first looks watery with blood and mucus in later stages. The intestine may look thickened and ballooned with red pinpoint lesions. *Eimeria tenella* affects the blind sacks of the gut. The intestine may be filled with blood and pus and turn in to a solid core.

Perez-Carbajal et al., (2010) detected that coccidia sporozoites infected intestinal epithelial lining resulted un tissue damage and trauma to the intestinal mucosa and sub mucosa.

Ahmed et al., (2012) mentioned that caceum of experimentally infected chickens with *E. tenella* showed different stages of coccidia, numbers of intracellular oocysts and severe hemorrhage. *E. necatrix* caused congestion of the intestinal muscularis.

Defar, (2017) performed postmortem examination in chicken infected with coccidia. Where, duodenum exhibited white lesions with hemorrhagic mucosa appearance. Jejunum possessed petechial hemorrhage; the jejunum thickened and ballooned with red pinpoint. Ileum appeared with thin intestinal

wall and hemorrhages. Caecum was thickened and ballooned, its content mixed with blood.

El-Katcha et al., (2018) noticed that small intestine infected with *E. acervulina, E maxima, E. mivati* and *E. tenella* was suffered from necrotic enteritis.

9- Coccidiosis economic impact:

Jordan, (1990) showed that in the last few years the poultry industry and as consequence chicken meat represents 80 percent of the whole production of meat originating from birds. Still, production is the fastest growing in the meat industry. According to analysis, production, as well as consumption of chicken meat, will rise because of good feed conversion in comparison of other animal species, there is not religious aspect of poultry meat consumption, poultry meat is healthy (low fat and high protein content) has good sensory qualities, low price and fast production which mean short generative time. Poultry, during coccidiosis and after therapy, have poor productive result. Daily feed quantity and feed conversion rise. Chicken daily growth weight is reduced, as well as body mass at the end of fattening period. As a result of fattening period should be prolonged. At the same time, care should be taken for withdrawal period for the drug which further rises costs of production.

Williams, (1998) mentioned that coccidiosis is recognized as the parasitic disease that has the characteristic economic impact on poultry production. The annual worldwide cost is evaluated about $800 million.

Vermeulen et al., (2001) displayed that coccidiosis control are still based on prophylactic medication via the feed and vaccination, not to exclude good production praxis and good hygiene and sanitation. Since in coccidiosis, carcass showed smaller yield, as well as the proportion of more valuable parts of the body.

10- Diagnosis procedure of coccidiosis:

Soulsby, (1982) mentioned that coccidiosis diagnosis in chicken is best detectable by postmortem changes of representative birds. Fecal examination led to quite erroneous results. The major pathological lesions are produced before oocysts are shed in the drooping as in *E. tenella* and, conversely, the presence of large number of oocysts indicated a serious pathogenic condition. Thus, with *E. acervulina*, which has a high biotic potential, comparatively larger numbers of oocysts are shed as in *E. necatrix*. Furthermore, the accurate identification of the *Eimeria* oocysts of various poultry coccidia is difficult.

10.1. Detection of oocyst in feces:

Conway and Mckenzie, (1997) found that oocysts in faeces of infected birds could be detected through floatation methods using saturated salt or sugar solution, since this method is not reliable for diagnosis of coccidiosis. It can be a useful in case of subclinical infection. Concentration floatation technique is used for the collection of *Eimeria* oocysts from intestinal content of chickens. *Eimeria* oocysts isolation depends on the measurements of oocysts by using a calibrated ocular micrometer at 400x magnification and location and characteristics intestinal lesion, oocyst morphology and sporulation time of *Eimeria* species.

10.2. Histopathology:

Reid, (1978) noticed that thickened intestinal mucosa or submucosa is due to parasitic invasion which is the more detectable during cutting, watch for. Mucus, blood, casts, or cores and cheesy coagulation necrosis was observed. Where, presence of blood in the caeca is indicator to *E. tenella*. But bleeding originate from the more anterior zones of the intestine and moving to the cecum may led to a misdiagnosis the case of *E. necatrix* as *E. tenella* infection. Because, differential diagnosis of histomoniasis, hemorrhagic syndrome and ulcerative and necrotic enteritis also produce somewhat is similar gross lesions.

Conway and McKenzie, (1991) noted that the observed lesions such as its intestinal tract location, its appearance and severity, the nature of intestinal contents and other associated gross change can be useful in establishing a diagnosis. The entire length of the external serosal surface of the digestive tract from the gizzard to the lower rectum displayed whitish plaques or petechiae. Whitish streaks or rounded colonies of oocysts in the duodenum are diagnostic to *E. acervulina* or *E. mivati*. In the mid gut area on both sides of the yolk sac diverticulum, whitish plaques may be produced by colonies of *E. necatrix* schizonts.

Vermeulen et al., (2001) showed that all *Eimeria* spp. localized in the intestinal epithelial lining induced extensive pathological alterations characterized by local destruction with intestinal necrosis.

Ahmed et al., (2012) observed hemorrhagic lamina propia, inflammatory cells aggregation, coagulative necrosis and intracellular oocysts in the middle part of intestine of experimentally infected balady chicks with *E. necatrix*. Also,

caceum of experimentally infected chickens with *E. tenella* showed different stages of coccidian, numbers of intracellular oocysts and severe hemorrhage.

Chapman, (2014) observed that pathogenicity of coccidia infection differs according species. Whereas, less pathogenic *E. mitis and E. acervulina* induced mild enteritis was noticed. Highly pathogenic *E. tenella and E. necatrix* caused the destruction of intestinal villi leading to hemorrhage and death.

11-Prevention and control of coccidiosis:

11.1. Chemotherapy:

Antibiotic ionophores such as salinomycin are directly considered cytotoxic for *Eimeria* spp. **(Conway et al., 1993)** and C. perfringens **(Engberg et al., 2000)**, therefore, it led to decrease parasitic and bacterial intestinal loads and reduce the corresponding host inflammatory responses. Alternatively, salinomycin may initiate anti-inflammatory pathways in the avian gut, as evidenced by increased transcription of the counter-regulatory cytokines IL-4 and IL-10, compared with *Eimeria* vaccination.

Urquhart et al., (1996) mentioned that control of coccidia in poultry could be done through the combination of good management and use of anticoccidial compounds in the feed or water. It was recommended that litter should be kept dry and special attention should be given to litter near water fonts or feeding troughs.

Chapman, (1997) displayed that most anticoccidial products possess biochemical effects upon a specific developmental stage of Coccidia. Broad categorization of the mode of action of anticoccidials on the parasite metabolism

has been undertaken. Traditional control through chemicals incase of coccidiostats are becoming less attractive due to increasing parasite resistance to chemicals. It can do through minimizing residues in the environment and in food, and the demands of alternative production systems (e.g. organic).

Williams, (1998) recorded that to reduce the effects of resistance, poultry producers rotate the use of various anticoccidials with successive flocks, combine chemical and ionophore treatments, or employ shuttle programs during a flock grow out. Treatment system depends on seasonal conditions and prevalence of various species of coccidia.

Chapman, (2001) & (2008) reported that the modern poultry industry have developed with the advent of drugs to control coccidiosis. Today, the prevention and control of coccidiosis depend hugely on chemotherapy and chemoprophylaxis using anticoccidial drugs.

Greif et al., (2001) showed that several of anticoccidial products influence essential biochemical pathways of the parasitic cell by affecting an important co-factor of named pathway.

Allen and Fetterer, (2002) found that anticoccidial drugs involving polyether ionophores can disrupt intracellular osmotic balance (e.g. salinomycin, monensin, lasalocid, and maduramycin) and chemicals which block metabolic pathways as amprolium, clopidol, decoquinate, and diclazuril.

Callaway et al., (2003) assessed that antibiotic ionophores exert a direct cytotoxic effect on coccidia parasites through their ability to facilitate the

transport of mono- and divalent cations via the cell membrane to toxic intracellular levels.

McDougald, (2003) revealed that the extensive use of the anticoccidial drugs has been a major factor in complement of the process of the industry for prevention and control of coccidiosis in poultry. This beneficial use of anticoccidial drugs is associated with a widespread drug resistance of coccidia in the United States, South America and Europe. The first line of defense against development of resistance is the use of shuttle programs (two or more drugs employed within a single flock) and frequent rotation of drugs (rotation of different compounds.

Kahn, (2005) noted that coccidiostats are considered the prophylactic drugs that used for prevention of coccidiosis. An effective role of the coccidiostat is to inhibit the schizogonic stage and allow immunity to develop. Prophylactic use is performed because most of the damage occurs before signs become apparent, and because drugs cannot completely stop an outbreak.

Babu et al., (2006); Li et al., (2010) showed that the relative impact of coccidiosis vaccination and in-feed salinomycin on serum levels of nitric oxide (NO) and specific antibodies, and on intestinal levels of cytokine transcript through reflection the heightened inflammatory status induced by the live parasites. Since, NO is produced by chicken monocytes and macrophages following exposure to enteric pathogens as salmonella, clostridium, and *Eimeria*. **Lee et al., (2011)** mentioned that infection with *Eimeria* protozoa also generates an antibody response specifically directed against the profilin protein, and up-regulates the expression of pro-inflammatory cytokines, while simultaneously down-regulating the expression of anti-inflammatory cytokines.

Taylor et al., (2007) showed that anticoccidial drugs run into two categories, the synthetic compounds popularly known termed chemicals that have specific modes of action against parasite metabolism, such as amprolium, clopidol decoquinate, halofuginone; and ionophore antibiotics, such as monensin lasalocid, salinomycin, narasin, and maduramycin, which act through general mechanisms of altering ion transport and disrupting osmotic balance

Gerhold et al., (2011) noted that usage either diclazuril or salinomycin reduced lesion score as compared with positive control group.

Hamad, (2011) mentioned that diclazuril treated birds had higher weight gain when in comparison with salinomycin treated ones. Also, FCR and mean body weights were improved in diclazuril treated quails when compared with control non treated quails and salinomycin treated ones.

Hameed et al., (2012) found that amprolium reduced mortalities in sulphadimidine sodium treated Japanese quails. Moreover, toltrazuril treated quails provided higher survival rates and lower mortalities comparing with positive control group.

El-Gaos, (2014) referred that cecal coccidiosis with *E. Tsunodai* infection given lowered clinical symptoms and mortalities in quails treated with diclazuril in comparison with salinomycin treated quails. Moreover, it recorded that both amprolium and ethopabate and toltrazuril treated groups showed oocyst output lower than that of positive control group. Amprolium and ethopabate treated groups shed fewer number of oocyst than toltrazuril treated one. While, body weights of the bird treated with amprolium and ethopabate was higher than that of toltrazuril (2.5%) treated one.

Sokol et al., (2014) recorded that toltrazuril lower sporulation percent compared with positive infected group. Also, it was found that toltrazuril caused a reduction in the percent of sporulated *E. tsunodai* oocysts in Japanese quails compared with positive control group.

El-Morsy et al., (2016) showed that the birds treated with diclazuril exhibited improved and noticeable results than coccidia infected birds resulted in lowered oocyst output in salinomycin treated group.

11.2. Vaccination:

Conway et al., (1993) noted that combined humoral and cellular immunity likely reflect host reactions not only to the live coccidia vaccine, but also to infectious *Eimeria* and Clostridium microorganisms found in the used litter on which the chickens were raised. Antibiotic ionophores such as salinomycin are directly possessing cytotoxic effect on *Eimeria*.

Shirley et al., (1995); Shirley and Bedrnik, (1997) displayed that the basic alternative to chemotherapeutic control of *Eimeria* is vaccination with live vaccines and it based upon immune protection induced by vaccination with oocysts containing different formulations of live wild-type or attenuated parasites of one or more species.

Danforth, (1998) reported that live oocyst vaccines are capable of a controlling subclinical infection early during grow-out for immunity development, where it decrease MBW gain and increased FCR in broilers when compared with medicated birds. The negative effects on cumulative broiler performance when using live oocyst vaccines compared with anticoccidial use,

was evidenced mainly by reduced final MBW. It could be concluded that vaccinated broilers have performed similarly to, if not better than, medicated broilers.

Williams et al., (1999) mentioned that vaccination program causing significantly lower mortality rates compared with medication system.

Yun et al., (2000) showed that vaccines strategies in the poultry industry have been tried for more than 50 years, primarily in broiler breeder and replacement layer flocks. Vaccine usage in the host develops immunity, affording the bird protection against subsequent infections by the same spp.

Vermeulen et al., (2001) displayed that a number of live anticoccidial vaccines, such as Coccivac®-B, Coccivac®-D, Immucox®-C1, Immucox®-C2, Paracox®, Paracox®-5, Livacox®-D, Livacox®-T and Livacox®-Q have been available in the world market for several years. These vaccines have contributed significantly for the prevention and control of chicken coccidiosis, although they have high effective role against clinical signs of avian coccidiosis. Worldwide usage of such vaccines, in particular live virulent vaccines, make it of limited use for broiler chickens because of the potential problem of transient slight fall in the weight gain after vaccination resulted in affecting broiler producers' confidence in using these vaccines for consideration of economic benefits.

Allen and Fetterer, (2002); Lillehoj et al., (2005) mentioned that part of the differential effects of vaccination versus pharmacologic medication on growth performance may be related to the different modes of action of these two disease management programs. Chickens infected with *Eimeria* develop protective immunity against re-infection by the homologous parasite. **Song et**

al., (2000); Ding et al., (2004) showed both cell-mediated immunity, by antigen-specific T lymphocytes and non-specific T cells and macrophages, and humoral immunity by parasite-specific antibodies have an important role in disease protection, although the relative contribution of antibodies remains debated.

Chapman et al., (2002) mentioned that live oocyst vaccination is constituted as a realistic alternative to the anticoccidial drugs for prevention of coccidiosis in broilers. There are four major brand of vaccines commercially available, and they are based on the use of wild type (Coccivac® D/B and Immucox®) and attenuated (Paracox® and livacox®) *Eimeria* species. The non-attenuated vaccines consisted of a mixture of oocysts of wild-type-strain *Eimeria* that will not produce pathogenic effect, but induce immunity.

Crouch et al., (2003) showed that vaccination is considered effective and safe alternate to control coccidiosis. Several commercial vaccines are being used to control coccidiosis in the different countries. Live oocyst vaccination is constituted an effective tool for the generation of immunity and protection against subsequent E. challenge manifested by increased MBW gain, reduced FCR, and reduced lesion development in vaccinated chickens in comparison with non-vaccinated chickens.

Dalloul and Lillehoj, (2005) mentioned that non-attenuated and attenuated vaccines are two types of coccidiosis vaccines which available to the poultry industry. The non-attenuated vaccines are consisted of mixtures of wild type strains of *Eimeria* that designed to provide the chicken with immunity without any pathogenic effects. Attenuated vaccines containing mixtures of strains that be chosen for reduced or no pathogenocity. Coccidiosis vaccines are

usually administered with the intention that the oocysts will be recycled in the litter then passed through the intestinal tract after the initial vaccination occurred. It provides the birds with the solid immunity available from proper vaccination procedure.

Volk et al., (2005) assessed that the body weight was higher in the medicated farms than vaccinated ones with significant difference at the end of the *Eimeria* cycle.

Suo et al., (2006) found that the average survival rate (95.28%) and FCR (91.98%) of vaccinated chickens were significantly higher than medicated chickens.

Olga et al., (2007) observed that vaccinated birds given characteristic body performance than medicated birds. On the other hand, **Williams et al., (1999)** found that FCR in medicated farm better than vaccinated farm.

Anwar et al., (2008) demonstrated that LivaCox® T recorded a noticeable protection in birds infected with the two different strains of *E. tenella*; the best protection was detected in birds infected with El- Behera strain. The resultant protection showed no mortalities in all of the VC subgroup (3) challenged by El- Behera strain and decrease mortalities in the VC subgroup (4) challenged by Matrouh strain; improved body weight, lesion scores, mucosal scrapings, dropping scores, and decreased oocyst counts in VC subgroups. The variation in protection obtained by using LivaCox® T against the two field strains of *E. tenella* observed that the use of local strains of *E. tenella* may give better protection. The use of local strains, rather than a commercial vaccine offered a protection against coccidiosis has previously been detected to be valid.

Lehman et al., (2009) found that medicated broilers manifested higher body weights compared with coccidia-vaccinated chickens during the first 3 weeks post-hatch. Where, chickens vaccinated with Coccivac-D given lowered weight gains and reduced weight gain to feed ratios when compared with salinomycin-fed birds within the first 3 weeks post-hatch. However, compensatory growth was detected in the immunized birds at later times, overall body weight gains at 8 weeks post-hatch remained higher in salinomycin-treated chickens. On the contrary, **Williams and Gobbi (2002)** reported that broilers taken a live attenuated coccidiosis vaccine exhibited greater body weights more than chickens that received an antibiotic growth promoter at 36-37 days (females) and 56 days (males) post-hatch.

Jenkins et al., (2010) showed that broilers infected with coccidia parasites isolated from poultry farms using live vaccination and treated with salinomycin provided higher weight gains compared with anticoccidial drugs-treated chickens. Salinomycin-fed group showed lowered body weight gains and emphasize the presence of drug-resistant *Eimeria* in the litter, leading to reduced drug susceptibility.

MATERIALS AND METHODS

Materials:

1- Experimental chicks:

Two hundred (200) one day old chickens obtained from commercial hatchery. It was equally divided into 5 groups each contain 40 chicks; all chicks are fed on ordinary ration free from any anticoccidial drug. All groups are kept under the same conditions and received the same procedures of management and vaccination program.

2- Ration:

The chicks will feed on prepared ration and the ration is devoid from any anticoccidial feed additive obtained from Nile Wady Company **(Table, 2)**.

composition	The ration		
	Starter	**Grower**	**Finishing**
Yellow corn	571	633	675
Soya bean oil	300	255	230
Bone meal	24.2	25.5	20
Corn glutin 60%	45	30	25
Sodium chloride	1.5	1.5	1.3
Fish meal	30	30	25
Mineral, vitamin mixture	3	2.7	1.1
L.D Methionine	1.2	1.2	0.5
L.Lysine	1.1	1.1	1.1
Total(Kg)	1000	1000	1000
Calculated analysis			
Total protein%	23%	20%	18%
Metabolizable	2990	3090	3175

3- Eimeria isolates:

Cecal Emeria isolate was obtained from the examined field in Qena Province, Egypt.

4-Anticoccidial drugs will used:

1-Amprolium sulphate 20%.

2-Diclazuril.

5-Vaccines:

The vaccines used in experiment:

1- Coccivac® -D (Intervet Schering-Plough Animal Health Pty Ltd.)

2-Hitchener IB, Holand) against newcastle disease was used on 7th day of age in drinking water.

1- IBD Blen.
2- Lasota (Intervet, Holand) for newcastle disease was used on 18th day in drinking water.

6-Chemicals used for preparation of oocyst inoculum:

1- Potassium dichromate 2.5% ($K_2Cr_2O_2$).

2- Saturated sodium chloride solution.

6-Chemicals used for histopathological examination:

1- Ethyl alcohol.

2- Hematoxyline and Eosin stain.

3- Formalin.

4- Xylene.

Methods:

Experimental design:

Two hundred (200) one day old chickens obtained from the commercial hatchery. It was equally divided into 5 groups each contain 40 chicks; all chicks are fed on ordinary ration free from any anticoccidial drug. All groups are kept under the same conditions and received the same procedures of management and vaccination program. The birds were divided into 5 groups (1, 2, 3, 4 and 5) as following:

Group (1): It was used as a control negative group (not infected and not vaccinated against coccidia and not receive any anticoccidial drugs).

Group (2): It was used as a control positive group (experimentally infected with Eimeria but not vaccinated against Eimeria, and not receive any anticoccidial drugs).

Group (3): It was vaccinated against Eimeria using-Coccivac®-D vaccine intraocular at 2nd day old.

Group (4): It was received amprolium as prophylactic anticoccidial drug (Amproxin 20% Pharma Sewde Company) 125 g/200 liters of drinking water (125 ppm Amprolium) for 7 days.

Group (5): It was received diclazuril as prophylactic anticoccidial drug. Add 50 ml per 200 liters of drinking water for 48 hrs (**DICLACOX Liquid** AVICO Company).

All groups were kept under daily observation for clinical signs, mortalities, with collection of droppings for oocysts calculation at 0,7,14 and

24^{th} days of age. Body weight and feed intake also were recorded for calculation of feed conversion rate for all the groups.

2-Preparation of Eimeria species inoculums and experimental infection:

a- Isolation of field Eimeria isolates from positive field infected cases with coccidiosis:

The two ceci of positive field infected cases are obtained and their contents were homogenized with water and sieved in a beaker through the pellet was resuspeded in potassium dichromate 2.5% $K_2Cr_2O_7$ in the presence of suitable humidity and temperature in a group of petri dishes.

The thickness of the fluid was not higher than 5 mm to facilitate the oxygen diffusion forced aeration was achieved (2-3 times daily) by removing the cover of petri dishes and shaking the suspension for few minutes. The plates were examined microscopically to assign the degree of sporulation, after sporulation occurs the sporulated oocysts were removed from fecal debris by series of centrifugation using NaCL (Centrifugation flotation technique). The suspension was centrifuged at moderate speed (1500 rpm) for 5-10 minutes to sediment the solids and allow oocysts to suspended at the top of supernatant. The floated oocysts were collected by Pasteur pipette and propagated **(Long et al., 1976)**.

B- Sporulation:

Fecal samples that contained abundant unsporulated oocysts were placed in medium sized petri dishes, forming a thin layer of liquid (~ 5mm) of 2.5% (w/v) aqueous potassium dichromate solution ($K_2Cr_2O_2$) and left at room temperature ($23\text{-}25^0$ c) to promote sporulation of oocysts. The oocysts were

repeatedly examined over a period of one week and the sporulation time was recorded **(Pandey et al., 1994)**.

C- Total oocyst count:

It was performed to achieve the intensity of infection by using McMaster technique according to **Abdel-Rahman, (1982)**.

Equipment:

1-Beakers
2- Balance
3-A tea strainer
4- Measuring cylinder
5-Stirring device (tongue depressor)
6- Pasteur pipettes
7-Flotation fluid
8- Microscope
9- McMaster counting chamber and tube

Procedure:

1. Accurately weighted 2 gm of fresh feces were suspended in 58 ml saturated sodium chloride solution.
2. Mix the contents thoroughly with a stirring device.
3. The largest particles were removed by straining the suspension via a fine tea strainer into container and the residues were pressed out.
4. While stirring the filtrate in container, take a sub-sample with a Pasteur pipette.
5. Fill both sides of the McMaster counting chamber with the sub-sample.
6. Allow the counting chamber to stand for 5 minutes.
7. Examine the sub-sample of the filtrate under a microscope at 10×10 magnifications.

8. Coccidian oocysts were counted within the engraved area of both chambers.
9. The number of oocysts per gram of feces can be calculated as follows:

$$\mathbf{X} = \frac{\text{Total no. of Oocyst}}{\text{Total no. of counting chambers}} \times 200$$

$\mathbf{X}$ = Oocyst per gram of feces

Or X = n x 200

N = the number of oocysts counted in one cell chamber.

D- Experimental infection:

About 5 $x10^4$ sporulated oocyst per bird were given orally by direct inoculation into the crop **(Vanparijs et al.,1989)** using rubber syringe after opening of the chick mouth and holding its neck backward **(Nada, 1980)**.

3-Sampling:

Fecal sample:

Representive fresh litter samples and cloacal swaps were collected, samples collected daily from the 5^{th} day after infection until 10 day post infection for oocyst count.

4- Evaluation of tested drugs and vaccination:

a- Clinical signs:

Description of the clinical coccidiosis in each group was diagnosed according to the parameters reported by **Vezey, (1970)**. The chickens after infection were observed for any clinical signs appeared.

b- Dropping scores:

The oocyst count was carried according to the method described by **Abdel-Rahman, (1982)**.

c- Lesion scores:

Recording of lesion scores was performed for the cecum involving the upper, middle and cecal portions of the intestine according to **Johnson and Reid, (1970)**.

d- Mortality rate:

The number of dead birds found in each group during the experimental period was recorded at the end of the experiment and calculated as a percent of the total birds and the exact cause of mortality was confirmed by postmortem examination.

e- Effect on chicken performance:

a- Weight gain:

The average weekly gains were evaluated by the difference between body weights of each two successive weeks for each group, according to the method described by **Hafez, (2008)**.

b- Feed consumption:

The amount of daily feed consumption of each group calculated by subtracting the remaining feed from the allowed daily amount then the average feed intake per chick daily and weekly was calculated for each group according to method described **by Hafez, (2008)**.

c- Feed conversion ratio:

Mean weight gain and FCR for each group were determined as described by **Holdsworth et al, (2004)**.

$$\textbf{FCR} = \frac{\text{Feed intake (gm) in a give period}}{\text{Body weight gain (gm) in same period}}$$

d- Statistical analysis:

Statistical analysis was done using one-way analysis of variance (ANOVA). It was done to compare between control and other treated groups, followed by post-hoc analysis (Dunnett's test) using SPSS (Statistical Package for Social Sciences) version 17 according to **Borenstein et al., (1997)**. The data were presented in form of Mean ± Standard Deviation. The difference was considered statistically significant when $P<0.01$.

e- Pathological examination:

At the end of experiment, all birds from each group will be scarified and observed for any gross changes. Concerning to the pathological sections, the intestinal parts are dissected. Then were collected, fixed in 10% formalin, dehydrated in absolute alcohol, cleared in xylene, and embedded in the paraffin wax for preparation of fine blocks; sections of 5 μm thicknesses were cut and subjected to routine hematoxylin and eosin staining **(Culling et al., 1985)**.

RESULTS

Clinical signs:

The positive control group showed a noticeable clinical manifestations represented by the poor performance and signs of inactivity, decrease feed intake, decrease in body weight in addition to bloody diarrhea and emaciation was noticed especially at 14, 21 and 24th days old chicks **(Fig. 1, a-c)**. On the contrary, the negative control group and the other treated groups, Coccivac-D vaccinated group, Amprolium, and Diclazuril group displayed an increment in body weight and body performance without any noticeable clinical signs.

Body weight, feed conversion rate and weekly gain:

The result of the body weight of the experimental groups/day/g and the and weekly gain/g was presented in **Table, 3; Figs. 2 & 3**. No significant differences were noticed in the experimental groups up to 14 days old chicks. **At 14th days old chicks**, the bird weight was significantly higher in the Vaccinated ($P<0.05$), Amprolium ($P<0.01$), and Diclazuril ($P<0.01$) group when compared with the positive group, and there was no significant difference in chicks weight between the negative control group and the other groups. **At 21th days old chicks**, no significant differences were noticed in the body weight of the negative control group and the vaccinated group, and between the positive control group and Diclazuril group. In addition, there was a significant decrement ($P<0.01$) of the body weight of the Amprolium group and the Diclazuril group when compared with the negative control group or the vaccinated group. **At 24th days old chicks**, the body weight of the negative control group was significantly higher ($P<0.01$) when compared with the other

experimental groups, while the diclazuril group showed a significant decrement ($P<0.01$) of the body weight when compared with the other experimental group. On the other hand, the vaccinated group showed a significant increment in the body weight ($P<0.01$) when compared with the positive control, Amprolium, and Diclazuril group. Subsequently, the treated and vaccinated groups recorded an improvement in the feed conversion rate attributed to an increase in the feed intake and weekly gain **(Table, 4; Figs. 3, 4 & 5)**.

Mortality rate and number of dead birds:

None of the experimental groups showed any mortality before the infection with coccidiosis, also the negative control group did not show any mortality until the end of the experiment, while the higher mortality rate was recorded in the positive control group that 19 out of 40 experimented birds were died represented 47.5% mortality rate. For the treated or vaccinated groups both drugs and the vaccine reduce the mortality rate among treated chicken when compared with the positive control group; the lower mortality rate was recorded in the vaccinated group that 2 chicks were died represented 5% mortality rate, followed by Amprolium group that 6 chicks were died represented 15 % mortality rate, and the higher mortality rate was recorded in the Diclazuril group that 7 chicks were died represented 17.5 % mortality rate (**Table, 5 & Figs. 6 & 7)**.

Oocyst counts per gram of feces (OPGC):

Oocyst counts per gram of feces (OPGC) after infection and percent reduction OPG or anticoccidial drugs and vaccine used among treated chicken groups were determined by the McMaster technique **(Table, 6 & Fig. 8)**. The

oocyst count detected a higher number in the control positive group. While, vaccinated group recorded a little oocyst count followed by amprolium group then diclazuril group.

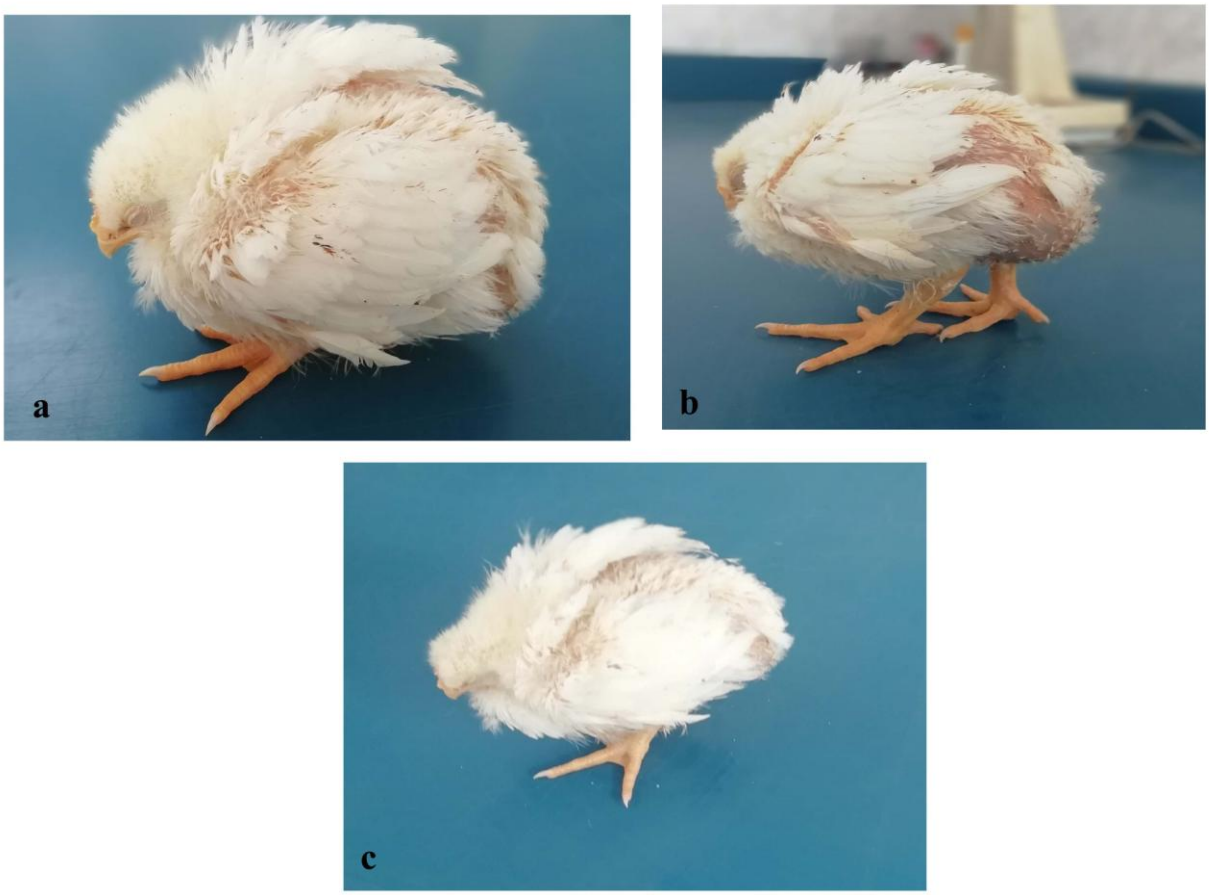

Fig. (1) a-c: Clinical signs of the control +ve group infected with *Eimeria tenella* showed noticeable clinical manifestations represented by poor performance, inactivity, decrease in the body weight and dropped feathers in addition to emaciation **(a, b & c)**.

Table (3): The mean values of average body weight and average weekly gain of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 0, 7, 14, 21 & 24th days.

Parameters / Groups	Average body weight/group/day/g					Average weekly gain				
	0	**7**	**14**	**21**	**24**	**0**	**7**	**14**	**21**	**24**
Control -ve	44.6± 0.2^{a}	195.4±2.7^{a}	359.5±8.6^{ab}	524.9±14.1^{a}	641.5±8.1^{a}	0	150.8	165.1	165.4	116.6
Control +ve	44.7±0.3^{a}	195.0±2.4^{a}	350.6±15.2^{a}	485.9±7.6^{b}	563.9±7.1^{b}	0	150.3	155.6	135.3	78.0
Vaccinated	44.2±0.2^{a}	195.7±2.7^{a}	364.4±15.3^{b}	518.3±11.7^{a}	628.1±3.8^{c}	0	151.5	168.7	153.9	109.8
Amprolium	44.4±0.2^{a}	197.1±2.3^{a}	372.3±19.5^{b}	506.6±7.6^{c}	590.7±5.6^{d}	0	152.7	175.2	134.3	84.1
Diclazuril	44.5±0.3^{a}	195.3±2.9^{a}	371.0±15.1^{b}	494.0±7.6^{b}	577.0±3.6^{e}	0	150.8	175.7	123	83

Data expressed as Mean ± SD
Each column, data followed by different letters is significant.

Table (4): The mean values of average feed intake and feed conversion of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 0, 7, 14, 21 & 24th days.

Parameters / Groups	Average feed intake				Average feed conversion				
	0	**7**	**21**	**24**	**0**	**7**	**14**	**21**	**24**
Control -ve	0	39	45.6	52.3	0	1.8	1.92	2.1	2.1
Control +ve	0	39	45.2	35.8	0	1.81	1.94	1.9	2.04
Vaccinated	0	39.1	45.7	50.2	0	1.8	1.8	2.28	2.1
Amprolium	0	39.2	46.3	40.4	0	1.79	1.85	2.1	2.1
Diclazuril	0	39.1	45.8	38.6	0	1.82	1.86	2.1	2.18

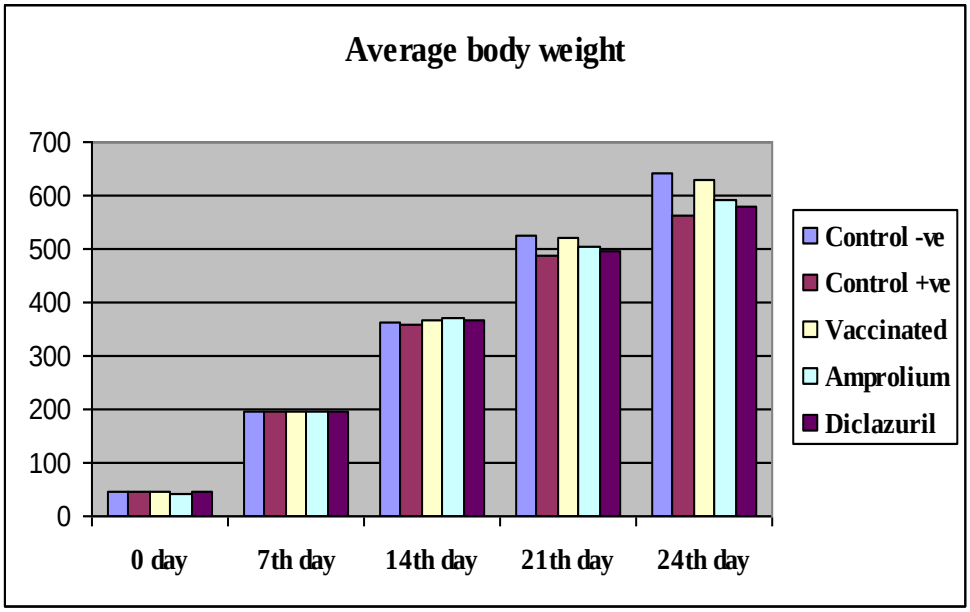

Fig. (2): The mean values of average body weight of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 0, 7, 14, 21 & 24th days.

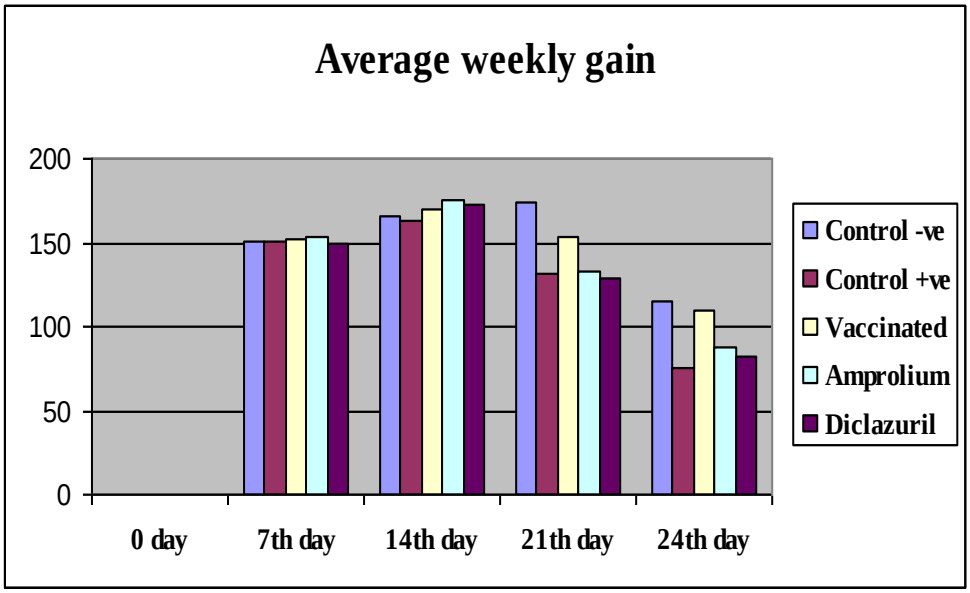

Fig. (3): The mean values of average weekly gain of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 0, 7, 14, 21 & 24th days.

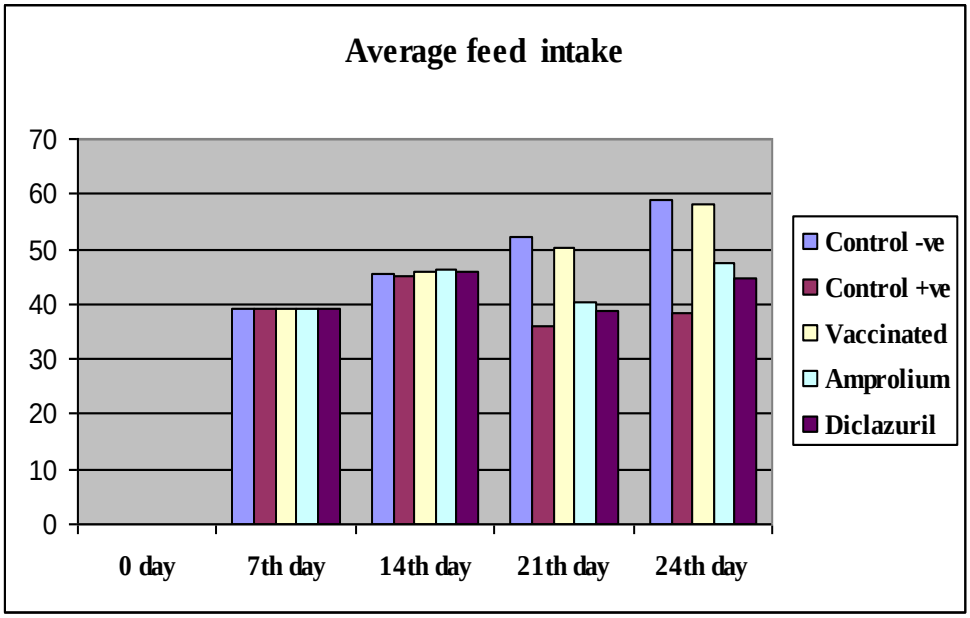

Fig. (4): The mean values of average feed intake of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 0, 7, 14, 21 & 24th days.

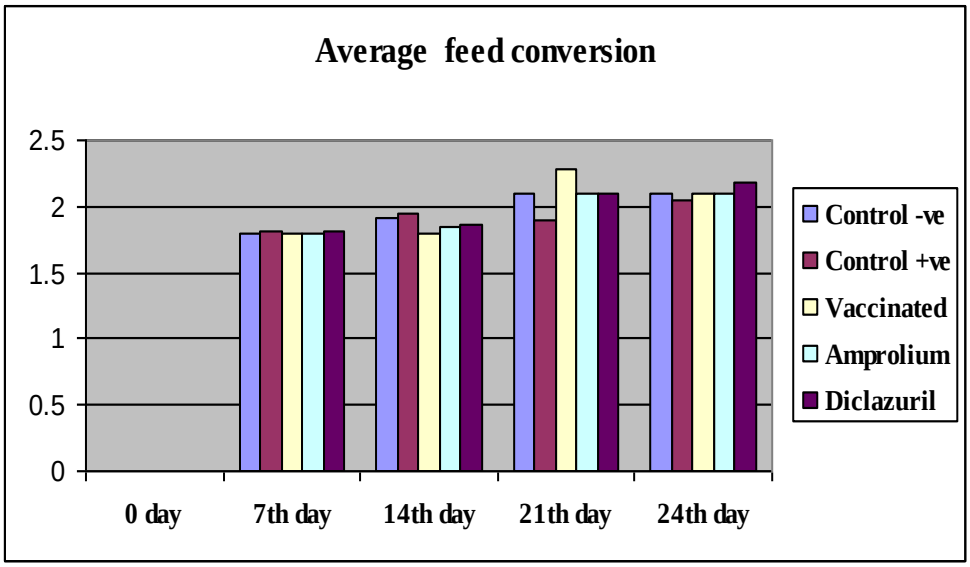

Fig. (5): The mean values of average feed conversion of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 0, 7, 14, 21 & 24th days.

Table (5): The effect of vaccine, amprolium and diclazuril on the number of dead chicken after infection with Eimeria and mortality rate in healthy and infected chicken with Eimeria.

The number of dead chicken after infection and mortality rate					
Groups / **Parameters**	**Group (1)**	**Group (2)**	**Group (3)**	**Group (4)**	**Group (5)**
	Control -ve	**Control +ve**	**Vaccinated**	**Amprolium**	**Diclazuril**
Total number of dead birds after infection	0	19	2	6	7
Mortality rate	0	47.5	5	15	17.5

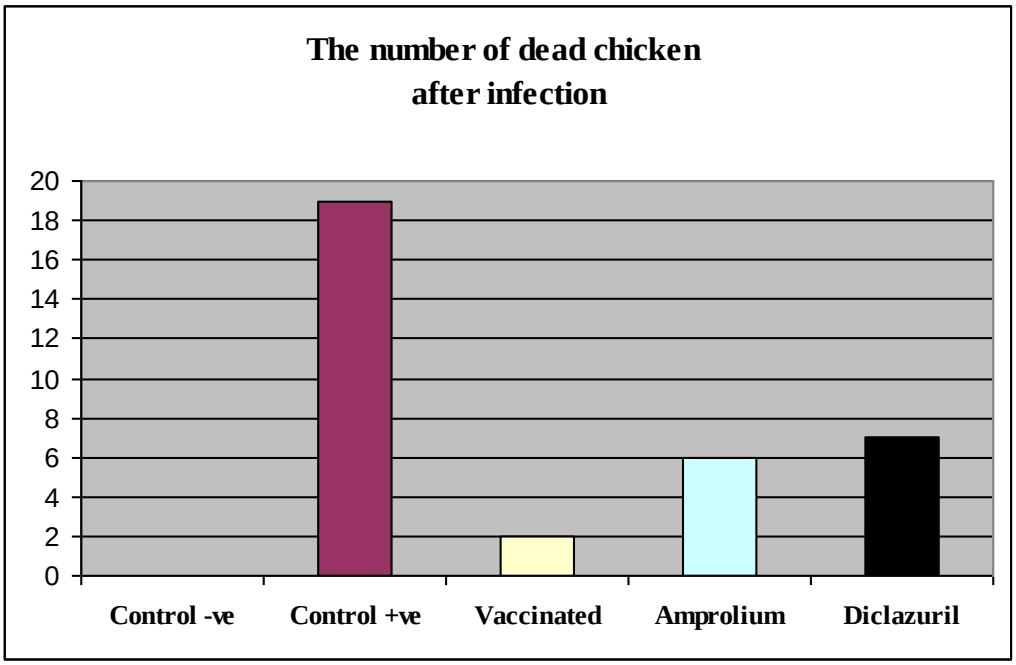

Fig. (6): The mean values of number of dead chicken after infection of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril).

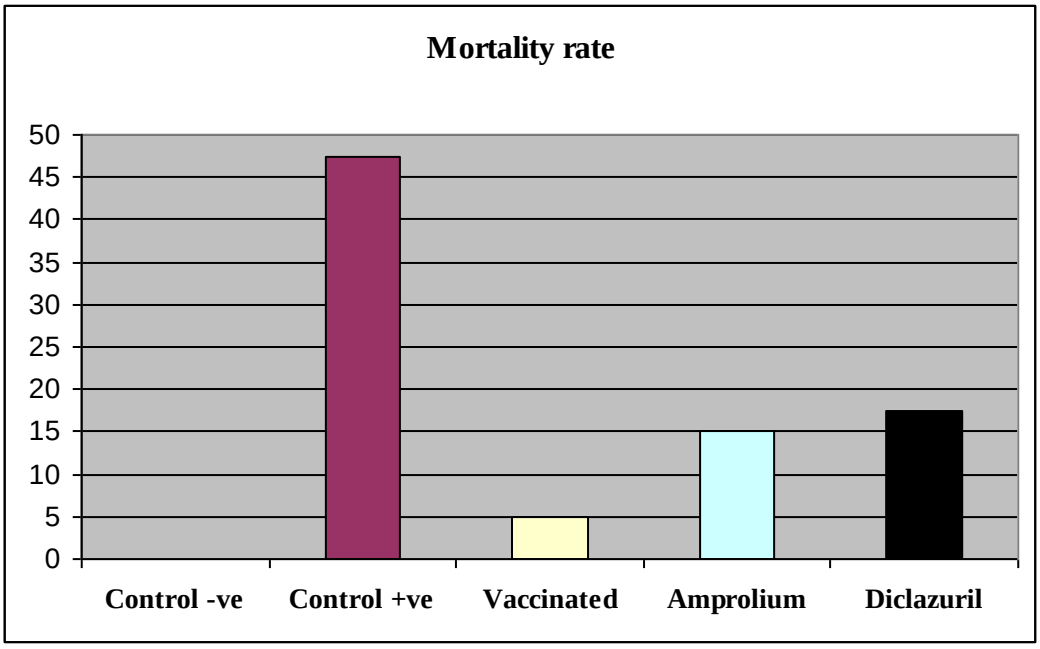

Fig. (7): The mean values of mortality rate (%) of group 1 (control -ve), group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril).

Table (6): Oocyst per gram (OPG) counts × 10^3/ days-old (day's post coccidial infection) for anticoccidial drugs and vaccine used among treated chicken groups.

Parameters / Groups	Oocyst per gram counts × 10^3/ days-old (days post coccidial infection)						
	18-days (4-days)	**19-days (5-days)**	**20-days (6-days)**	**21-days (7-days)**	**22-days (8-days)**	**23-days (9-days)**	**24-days (10-days)**
Control +ve	107±3.0^a	119.3±9.0^a	123±3.0^a	129.7±4.0^a	133.1±3.0^a	145.2±5.0^a	167.6±3.0^a
Vaccinated	34.5±4.0^b	36.3±6.0^b	29.7±5.0^b	21.4±2.0^b	17.6±3.0^b	11.5±40^b	2.1±0.6^b
Amprolium	2.3±0.7^c	8.5±2.0^c	10.2±2.0^c	17.2±3.0^b	21.3±4.0^b	29.8±3.0^c	35.7±3.0^c
Diclazuril	3.4±0.4^c	11.3±2.0^c	17.3±3.0^d	21.3±4.0^b	37.4±3.0^c	55.2±3.0^d	63.0±3.0^d

Data expressed as Mean ± SD

Each column, data followed by different letters is significant.

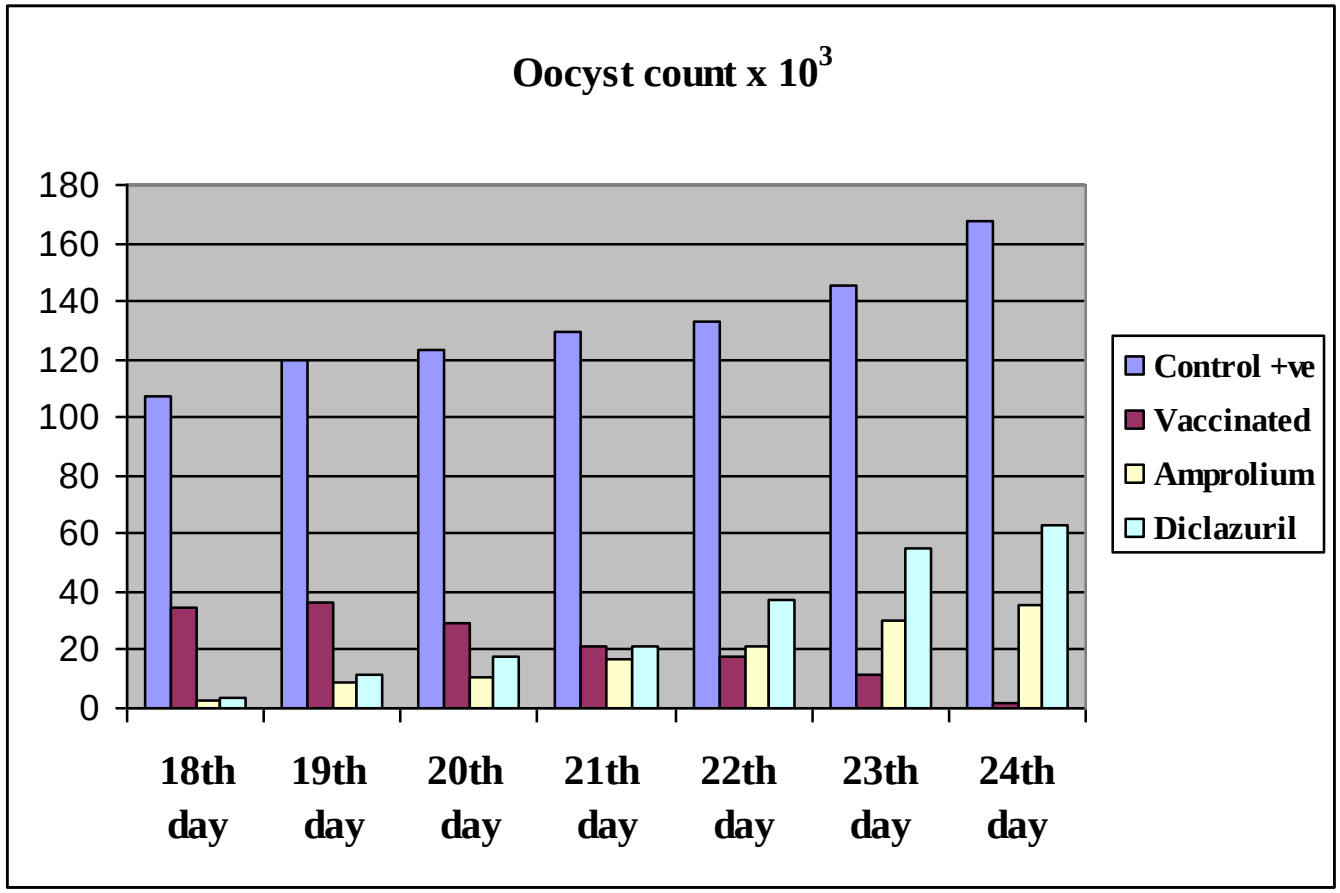

Fig. (8): The mean values of the oocyst count x 10^3 of group 2 (control +ve), group 3 (vaccinated), group 4 (amprolium) and group 5 (diclazuril) at 18, 19, 20, 21, 22, 23 and 24^{th} day after infection.

PATHOLOGICAL RESULTS:

1- Macroscopically (Grossly):

The control negative group exhibited normal appearance of the intestine. While, the Eimeria infected group showed severe hemorrhage and congestion of the intestinal tissues, where intestine appeared engorged and dilated with blood oozing abundant blood when cut. Also, Eimeria infected group displayed friable intestinal tissues wall. On the contrary, vaccinated group showed normal architecture of the intestinal tissues. Amprolium and diclazuril treated groups exhibited mild congestion of the intestinal blood vessels, besides slight thickening in the intestinal wall **(Fig. 9 a-f)**.

2- Microscopically:

The intestine of the group 1 (control negative) detected normal intestinal layers involving mucosa and submucosa, with normal muscular and serosa layers **(Fig. 10 a-d)**.

The intestine of the group 2 infected with Eimeria showed heavily infiltrated with different developmental stages of Eimeria involving oocysts, microgametes and macrogametes **(Fig. 11 a-d)**. Also group 2 recorded remarkable pathological changes involving extensive necrosis with sloughing of intestinal villi **(Fig. 12 a & b)**, besides highly destruction and lyses of the intestinal tissues including glands **(Fig. 12 c, d & e**), with severe congestion and dilatation of the blood vessels with perivascular inflammation **(Fig. 12 f)**.

Group 3 which vaccinated against Eimeria detected minimally infiltrated Eimeria oocysts with mild sloughing of the intestinal villi **(Fig. 13 a & b)**,

intestine appeared mildly infiltrated with inflammatory cells **(Fig. 13 c)**. Other cases displayed normal histological structure of the intestinal tissues and glands **(Fig. 13 d)**.

Group 4 (amprolium treated group) showed moderately infiltrated Eimeria oocysts with apparently normal the intestinal tissues **(Fig. 14 a & b)**. There was slight congestion and dilatation of the blood vessels **(Fig. 14 c)**. Also, mild sloughing of the intestinal epithelium with mild inflammatory cells was noticed **(Fig. 14 d)**, in addition to normal intestinal glands **(Fig. 14 e)** with normal intestinal villi **(Fig. 14 f)**.

Group 5 (diclazuril treated group) revealed some Eimeria oocysts embedded among intestinal tissues and glands **(Fig. 15 a & b)**. There was a moderate degree of necrosis of the intestinal epithelium **(Fig. 15 c)**, additionally necrosis of the intestinal tissues and glands was recorded **(Fig. 15 d & e)**, mild congestion and dilatation with peri- vascular inflammatory cells **(Fig. 15 f)**. Moreover, there was a moderate degree of thickness of the intestinal wall. There was highly infiltration with red eosinophilic substances and fluids toward intestinal lumen.

Comparative figure **(Figs. 16 a-j)** of the intestine of the group 1 showed normal architecture of the intestine **(Fig. 16 a & b)**, group 2 (Eimeria infected group) showed extensive necrosis and destruction of the intestine **(Fig. 16 c & d)**, group 3 (vaccinated) showed apparently normal structure of the intestine **(Fig. 16 e & f)**, group 4 (amprolium) showed moderately infiltrated coccidia oocysts **(Fig. 16 g)** with apparently normal intestinal tissues and glands **(Fig.16 h)**, group 5 (diclazuril) showed moderated degree of necrosis of the intestine **(Fig. 16 i & j)**.

The histopathological score of the intestine of G. 1 (control), G. 2 (Eimeria), G. 3 (vaccinated), G. 4 (amprolium) and G. 5 (diclazuril) stained with Hematoxylene and eosin were classified according to severity into severe (+++), moderate (++), mild (+) and absent (-). Whereas, group (2) exhibited severe gross lesions and remarkable pathological changes, on the contrast, vaccinated and treated groups revealed either moderate or mild pathological changes **(Table, 7)**.

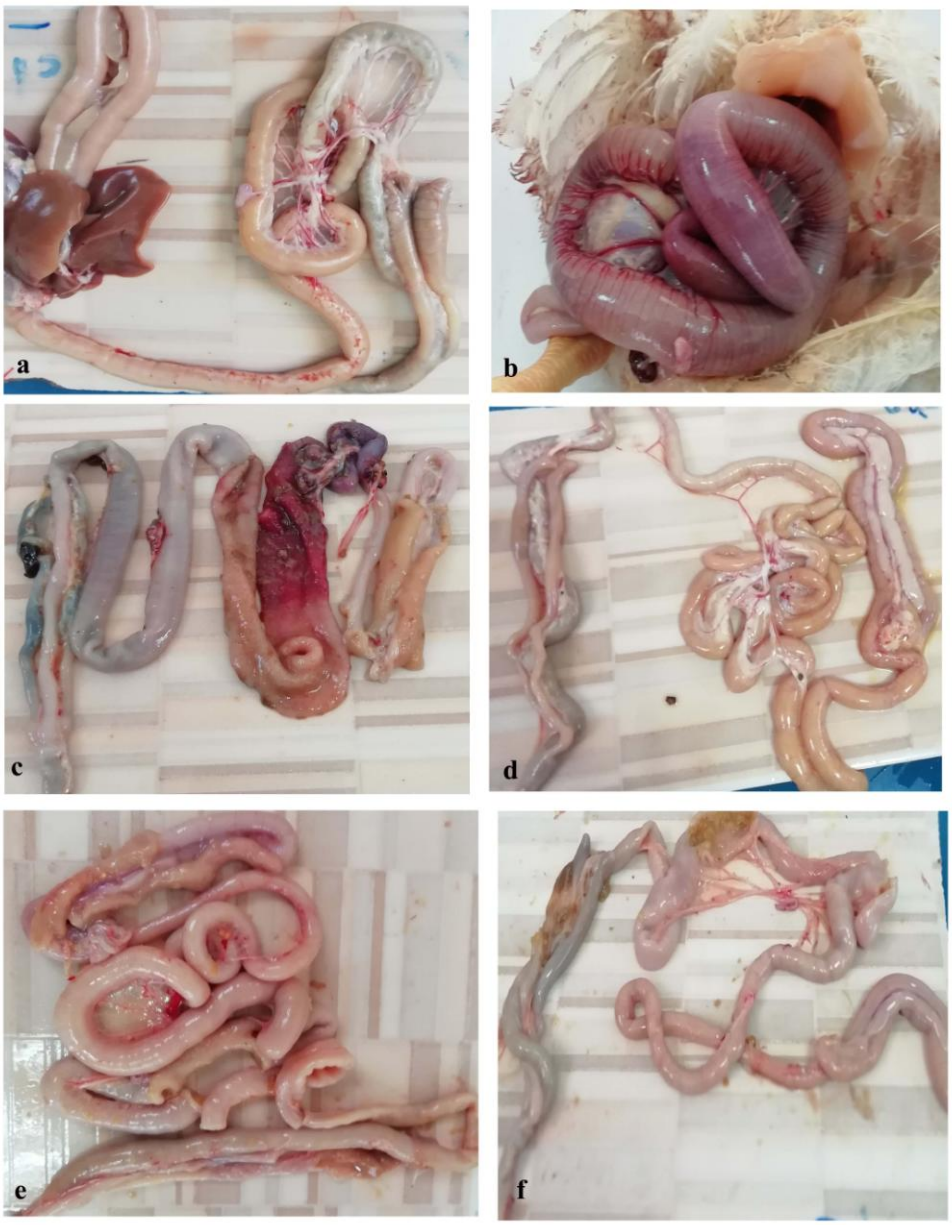

Fig. 9 (a-f): Gross lesions of the intestine of the group (1) showing normal intestine view **(a)**, group (2) with severe hemorrhage and bloody intestinal contents **(b & c)**, group (3) showing intact intestinal appearance **(d)**, and groups (4 & 5) with slight hemorrhage **(e & f)**, respectively.

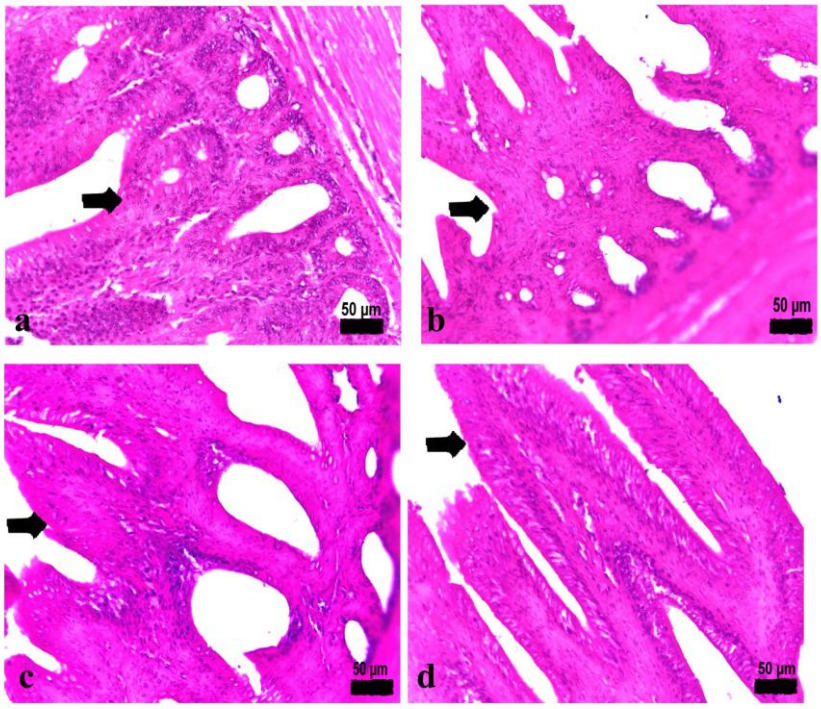

Fig. 10 (a-d): Photomicrograph of the intestine of the group 1 (control negative) showing normal intestinal structure involving intact intestinal glands, and villi with normal muscular and serosa layers. **(H&E., Bar= 50 µm)**

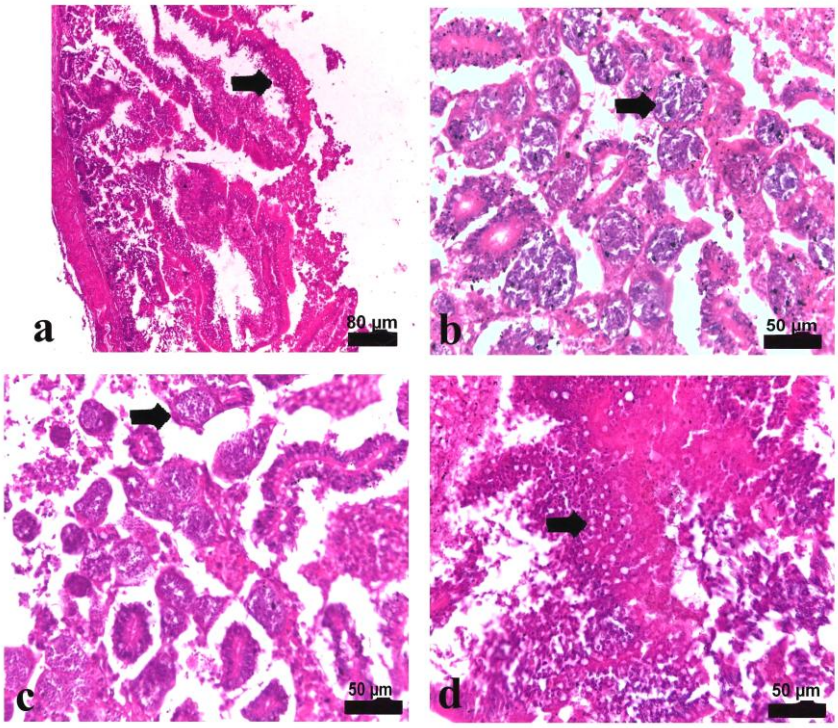

Fig. 11 (a-d): Photomicrograph of the intestine of the group 2 (control positive) which infected with coccidian showing heavily infiltrated Eimeria stages with oocysts, microgametes and macrogametes.

(H&E., Bar= 50 & 80 µm)

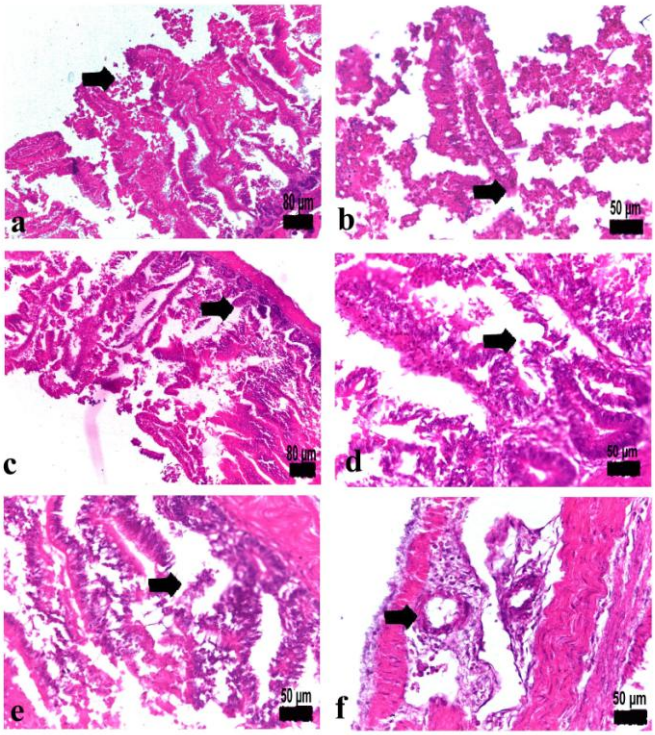

Fig. 12 (a-f): Photomicrograph of the intestine of the group 2 (control positive) which infected with coccidia showing extensive necrosis with sloughing of intestinal villi **(a)**, high power of **Fig a** showing extensive necrosis with sloughing of intestinal villi **(b)**, highly destruction and lyses of the intestinal glands **(c**), high power of **Fig c** showing severe destruction and lyses of the intestinal glands **(d & e)**, severe congestion and dilatation of the blood vessels with perivascular inflammation **(f)**.

(H&E., Bar= 50 & 80 µm)

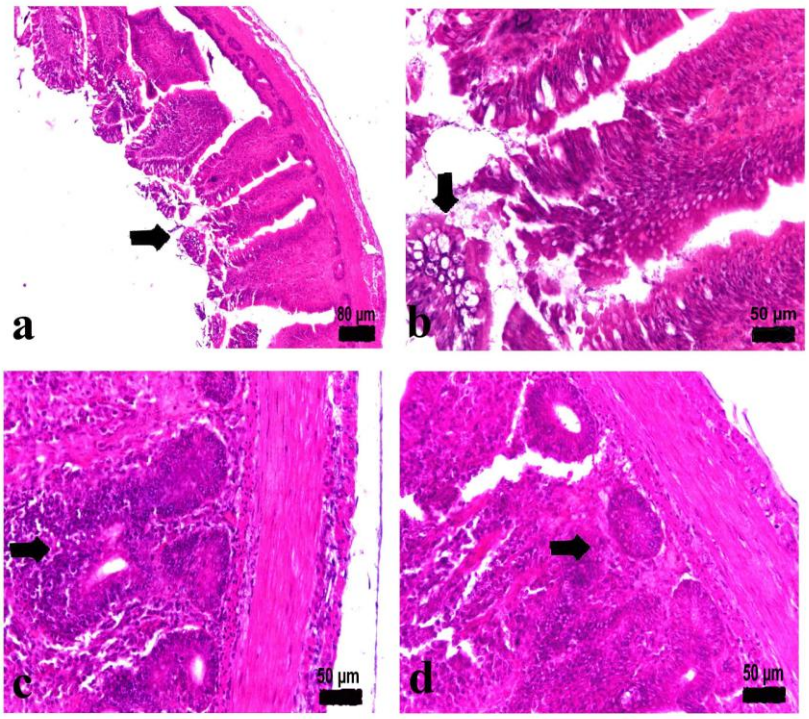

Fig. 13 (a-d): Photomicrograph of the intestine of the group 3 (vaccinated against Eimeria) showing minimally infiltrated Eimeria oocysts with mild sloughing of the intestinal villi **(a)**, high power of **Fig. a** showing minimally infiltrated Eimeria oocysts with mild sloughing of the intestinal villi **(b)**, mild infiltration with inflammatory cells **(c)**, apparently normal intestinal tissues and glands **(d)**.

(H&E., Bar= 50 & 80 µm)

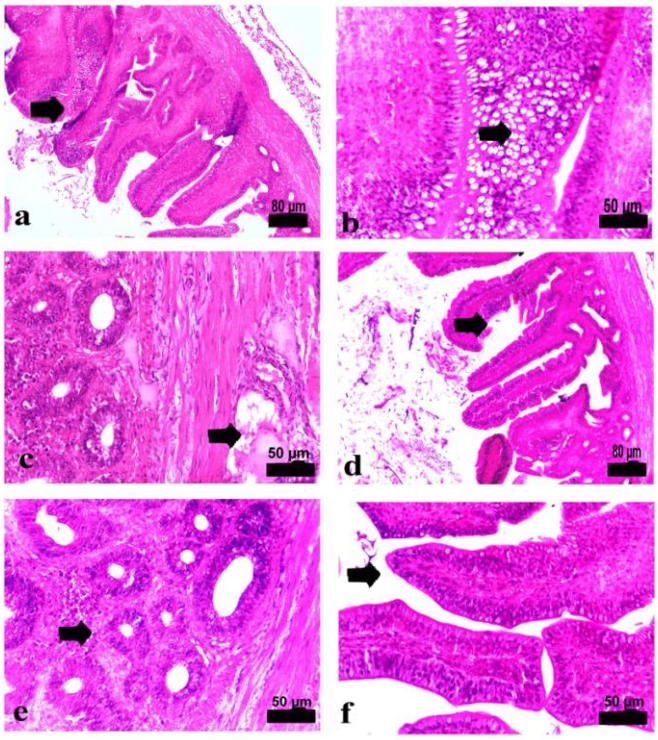

Fig. 14 (a-f): Photomicrograph of the intestine of the group 4 (amprolium treated group) showing moderately infiltrated Eimeria oocysts with apparently normal the intestinal tissues **(a)**, high power of **Fig. a** showing moderately infiltrated Eimeria oocysts **(b)**, slight congestion and dilatation of the blood vessels **(c)**, mild sloughing of the intestinal epithelium with mild inflammatory cells **(d)**, normal intestinal glands **(e)** with normal intestinal villi **(f)**.

(H&E., Bar= 50 & 80 µm)

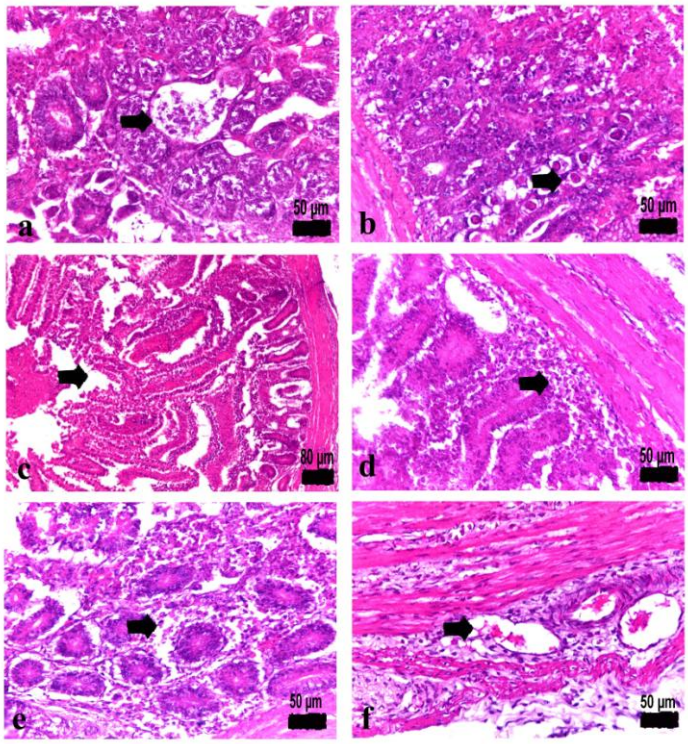

Fig. 15 (a-f): Photomicrograph of the intestine of the group 5 (diclazuril treated group) showing Eimeria oocysts embedded among intestinal tissues and glands **(a & b)**, degree of necrosis of the intestinal glands **(c)**, moderate necrosis of the intestinal tissues and glands **(d & e)**, mild congestion and dilatation with peri-vascular inflammatory cells **(f)**.

(H&E., Bar= 50 & 80 µm)

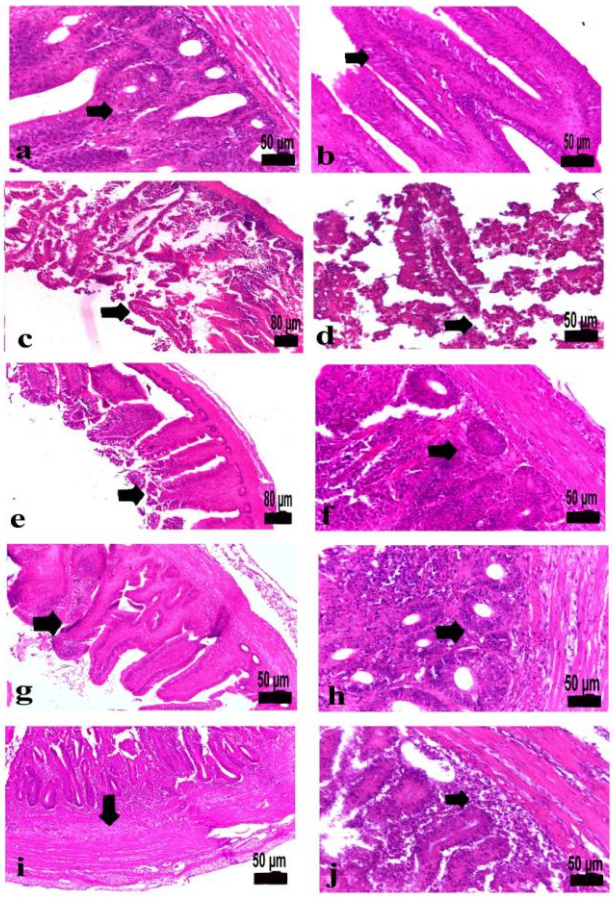

Fig. 16 (a-j): Comparative figure of the intestine of the group 1 (control negative) showing normal architecture of the intestine **(a & b)**, group 2 (Eimeria infected group) showing extensive necrosis and destruction of the intestine **(c & d)**, group 3 (vaccinated) showing apparently normal structure of the intestine **(e & f)**, group 4 (amprolium) showing moderately infiltrated coccidian oocysts **(g)** with apparently normal intestinal tissues and glands **(h)**, group 5 (diclazuril) showing degree of necrosis of the intestine **(i & j)**.

(H&E., Bar= 50 & 80 µm)

Table (7): The histopathological score of the intestine of G. 1 (control), G. 2 (Eimeria), G. 3 (vaccinated), G. 4 (amprolium) and G. 5 (diclazuril) stained with Hematoxylene and eosin were classified according to severity into severe (+++), moderate (++), mild (+) and absent (-).

Lesions \ Groups	G. (1)	G. (2)	G. (3)	G. (4)	G. (5)
Macroscopically (Grossly)					
Hemorrhage and congestion of the intestine	-	+++	+	++	++
Engorgement and dilated with bloody content	-	+++	-	+	+
Oozing of the blood when cut	-	+++	-	+	+
Thickening of the wall	-	-	-	++	++
Friable intestinal wall	-	+++	-	+	+
Microscopically					
Eimeria oocysts and stages	-	+++	+	++	++
Necrosis of the intestinal epithelium	-	+++	+	+	+
Sloughing and desquamation of the villi	-	+++	+	+	++
Inflammatory cells infiltration	-	++	+	+	+
Thickening of intestinal wall	-	-	-	++	++
Congestion and dilatation of blood vessels	-	+++	+	++	++

Absent (-), Mild (+), Moderate (++), and severe (+ ++)

DISCUSSION

Avian coccidiosis is a disease caused by one or moreof Eimeria species and considered as one of the most economically important and common diseases in spite of the advancement of chemotherapy, biosecurity, nutrition, or genetics **(Mcdougald and Raid, 1991)**. The economic losses due to coccidia are not limited to impaired growth, poor food utilization and depigmentation but it might cause a metabolic change in the tissue composition and dietary requirement, all of which impact adversely on poultry production **(Allen, 1986)**. Anticoccidial drugs or vaccination using live oocysts elicited significant protection against coccidiosis in chicken. So, the present study was constructed to compare the relative effectiveness of two disease control drugs involving Amprolium and Diclazuril and vaccination using Coccivac D, live oocyst vaccine.

In the present study, we noticed that the positive control group showed a noticeable clinical manifestation represented by poor performance and inactivity signs, decrease in feed intake, decrease in the body weight, ruffled and dropped feathers, and featherless areas bloody diarrhea and emaciation was also noticed especially at 14, 21 and 24th days of the experiment, these findings agreed with the results of previous studies reported by **Allen and Fetterer, (2002); Guo et al., (2007); McDougald and Fitz-Coy, (2008); Taylor et al., (2007)**. On the contrary, the negative control, vaccinated and treated groups (Amprolium and Diclazuril) displayed a good health status manifested by the higher in the body weight and the body performance with good activity when compared with the positive control group. This proves the correlation of the existence of cecal lesions caused by Eimeria and the subsequent malabsorption of nutrients,

anorexia, and listlessness of infected chick as mentioned by **Gautam et al., (2005)**.

Regarding the mortality rate in our study, we recorded a high mortality rate among the positive control group (47.5%). The vaccinated group showed the lower mortality rate (5%) when compared with the Amprolium (15%) and Diclazuril (17.5%) group, the results of mortality rate, agree with those recorded by **Williams et al., (1999)** who found that the losses from the vaccinated birds totaled 7.0% and those from the medicated birds 7.6 and other studies **(Bushell, 1992; Bushell et al., 1990; Shirley et al., 1995; Williams and Gobbi, 2002)** which found that vaccinated broilers have significantly lower mortalities than birds treated with anticoccidial drugs. Live anticoccidial vaccines are evidenced to be an effective alternative to anticoccidial drugs for the prevention and control of chicken coccidiosis **(Amal Kumar Sarkar, 2006; Williams et al., 1999)**. These vaccines have shared significantly in the control of chicken coccidiosis **(Vermeulen et al., 2001; Williams, 2002)**. The basis for vaccine program is depending on immunity that develops in the host, affording the bird protection against subsequent infections by the same spp. **(Yun et al., 2000)**.

The result of the body weight of the experimental groups showed no significant differences between the experimental groups up to 14 days old chicks, and all the differences were noticed after challenge with coccidia at 14^{th} days old chicks. After the challenge with coccidia, all groups were significantly lower than the negative control group until the end of the experiment. It was important to compare between the overall body weight of vaccinated, Amprolium and Diclazuril groups. The body weight of the diclazuril group was significantly lower ($P<0.01$) than the other experimental group, while the body

weight of the vaccinated group was significantly higher (P<0.01) when compared with positive control, Amprolium, and Diclazuril group, and this proves that the vaccination against coccidia gave a better results than the prophylactic effect of Amprolium or Diclazuril and these results supported the results of **Rashid et al., (2012)** who prove the prophylactic effect of vaccination on body weight gain and on preventing the coccidial infection.

In the current study, the modified McMaster method was used to compare the OPGC of the vaccinated, Amprolium and Diclazuril group. The OPGC of the Vaccinated group was significantly higher (P<0.01) than the Amprolium and the Diclazuril group at 18-20 days old chicks (4-6 days post coccidial challenge), while at 7-days post coccidial challenge no significant difference was noticed between the vaccinated, Amprolium and Diclazuril group. At 23-24 days old chicks (9-10 days post-infection) the Vaccinated group showed a significant decrement (P<0.01) in the OPGC when compared with the Amprolium and Diclazuril group. The overall result of the OPGC along the experimental days (4-10 days post coccidial challenge) showed that the positive control, Amprolium, and Diclazuril group had a continuous increment in the OPGC by time up to the end of the experiment, while the vaccinated group had a continuous decrement in the OPGC by time. The results of oocysts count agreed with the results recorded by **Williams et al., (1999)** who found that the patterns of mean oocysts counts in the litter vaccinated birds produced a rapid build-up of oocysts peaking at 21 days. Medicated birds produced a rather slower build-up with a single peak at 35 days with higher numbers remaining than numbers in the vaccinated crops and also in accordance with **Suo et al., (2006)** who found that from 11 to 20 days the peak of oocysts production were observed in each house during the experiment in immunized chickens, and samples from medicated birds showed irregular curves with oocysts numbers higher than of

vaccinated ones after this period, because anticoccidial drugs (Diclazuril and Toltrazuril) were used to control clinical coccidiosis. On the contrary, our results disagree with that recorded by **Williams and Gobbi, (2002)** who found that in all farms of vaccinated birds there was a major peak of oocysts numbers in litter at 27 days, with a shoulder at 34 to 36 days, somewhat suggestive of a second surge of oocysts production that had been rapidly brought under control by the birds immunity, indicating that the faster developing precocious lines contributed to at least the earlier portion of the peak in vaccinated birds. The late shoulder on this peak coincident with the maximum oocysts counts in anticoccidial drug-treated birds. It is notable that the litter oocysts concentrations for the birds treated with anticoccidial drugs were much lower than those for vaccinated birds.

In our study, the normal appearance of the intestinal wall was noticeable in both negative control and the vaccinated group. While, the positive control group showed the typical coccidial lesions, and these lesions were milder in both Amprolium and diclazuril treated groups and these results were a consequence of the tissue damage and trauma to the intestinal mucosa and submucosa which resulted from the different stages of coccidian life cycles within the intestinal mucosa **(Al-Gawad et al., 2012; Defar, 2017; Perez-Carbajal et al., 2010)**.

In the herein study the histopathological lesions of the intestine were examined to compare the effect of *Eimeria* on the intestinal line of the different experimental groups. The intestine of the negative control showed normal architecture and normal histological lining of the mucosa, submucosa, muscularis, and serosa. On the contrary, the intestine of the positive control group showed the typical lesions of coccidial infection which agreed with the

results of a previous study observed by **Vermeulen et al., (2001)**. The severity of the lesions also reflected on the OPGC of the challenged *Eimeria* which was significantly higher ($P<0.01$) than the other experimental groups along the seven days of counting and up to the end of the experiment.

The histopathological lesions of Vaccinated, Amprolium and Diclazuril group was approximately the same, but the Vaccinated group showed milder lesions when compared with the Amprolium and Diclazuril groups, the Vaccinated group was the minimally infiltrated group with *Eimeria* oocysts and reveal a mild sloughing of the intestinal villi, intestine appeared mildly infiltrated with inflammatory cells. Other cases displayed the normal histological structure of the intestinal tissues and glands. On the other hand, the pathological lesions of both the Amprolium and Diclazuril group were moderate but more severe than the vaccinated group, and this reflected on the OPGC. The diclazuril group showed moderate lesions and the OPGC was significantly higher ($P<0.01$) followed by the Amprolium group ($P<0.01$) then followed by the Vaccinated group ($P<0.01$). Those results supported by **Vermeulen et al., (2001)** who explain that the severe pathological changes with intestinal necrosis and desquamation of the epithelial lining were observed among *Eimeria* infected chicks in addition to congestion and dilatation in the blood vessels of the intestine. In addition, **McDougald and Fitz-Coy, (2008)** detected the liberation, the penetration activity of sporozoites to the epithelium of the intestine, and then the transportation in macrophages through the lamina propria of the villi to reach the epithelium at the depth of the intestinal glands, where further developments occur. *Eimeria* showed acceptable numbers of oocyst in lamina propria of cecum in addition to severe hemorrhage with extensive desquamation of epithelium and edema of muscular tissue and these were similar to **Perez-Carbajal et al.,**

(2010) who detected that coccidia sporozoites infected the cells of the intestinal lining caused tissue damage and trauma to the intestinal mucosa and submucosa.

A usage of anticoccidial drugs either amprolium or diclazuril offered significant results, but not better than in vaccinated birds. Anticoccidial drugs affect biochemical pathways that are dependent upon an important cofactor. Where, amprolium competitively prevent the uptake of thiamine by the parasite. Also, it hinder energy metabolism in the cytochrome system of the *Eimeria*. Quinolones and clopidol inhibit electron transport in the parasite mitochondrion, but by different pathways. Ionophores are capable to form lipophylic complexes with alkaline cations as Na^{+}, K^{+}, and Ca^{++} and transport these cations through the cell membrane and then affect a range of processes that based upon ion transport, such as influx of sodium ions thus, leading to severe osmotic damage. These drugs act against the extracellular stages of life cycle of the *Eimeria* **(McDougald, 2003; Chapman, 1997)**.

SUMMARY

Coccidiosis is defined as a widespread parasitic disease with the severe economic influence on poultry production. Infection with coccidian parasites leads to economic losses resulting from malabsorption of the nutrients. It resulted in remarkable pathological changes with severe destruction of the intestinal mucosa. Application of anticoccidial drugs or uses of vaccination with live oocysts could offer a significant protection against coccidiosis.

The present study was established on two hundred numbers of one day old chickens obtained from commercial hatchery. It was equally divided into 5 groups each contain 40 chicks; all chicks are fed on ordinary ration free from any anticoccidial drug. All groups are kept under the same conditions and received the same procedures of management and vaccination program. The birds were classified into 5 groups (1, 2, 3, 4 and 5) as following: Group (1), it was used as a control negative group (not infected and not vaccinated against coccidia and not receive any anticoccidial drugs). Group (2), it was used as a control positive group (experimentally infected with Eimeria but not vaccinated against Eimeria, and not receive any anticoccidial drugs). Group (3), it was vaccinated against Eimeria tenella using-Coccivac® -D vaccine intraocular at 2nd day old. Group (4), it was received amprolium as prophylactic anticoccidial drug (Amproxin 20% Pharma Sewde Company) 125 g/200 l of drinking water (125 ppm Amprolium) for 7 days. Group (5), it was received diclazuril as prophylactic anticoccidial drug. Add 50 ml per 200 liters of drinking water for 48 hrs (DICLACOX Liquid AVICO Company).

All groups were kept under daily observation for clinical signs, mortalities, with collection of fecal droppings for oocysts calculation at 0,7,14

and 24 days of age. Body weight, feed intake and feed conversion rate also were recorded for feed conversion rate calculation for all the groups. Moreover, gross lesions and histopathological findings were assessed.

The results proved noticeable clinical manifestations among control +ve group infected with *Eimeria tenella* represented by poor performance, inactivity, decrease in the body weight and dropped feathers in addition to emaciation. Vaccinated and treated groups showed higher in the body weight and the body performance with good activity in comparison with control +ve group.

Feed conversion rate and weekly gain was remarkably decreased among control +ve group attributed to decrease in feed intake. Other groups either control -ve or treated groups exhibited an improvement in Feed conversion rate and weekly gain. Higher mortality rate recorded among *Eimeria tenella* infected group. While, other groups involving drugs and the vaccine detected reduction in the mortality rate in comparison with control +ve group.

The oocyst count detected higher number in control positive group. While, vaccinated group recorded little count in the oocyst count followed by amprolium group then diclazuril group.

Gross lesions of the intestine of the control -ve group showed normal intestine view, control +ve group with severe hemorrhage and bloody intestinal contents, vaccinated group showed intact intestinal appearance, and amprolium and diclazuril group had slight hemorrhage. Histopathologically, *Eimeria tenella* infected group induced severe pathological alterations characterized by extensive necrosis and destruction of the intestine. A use of anticoccidial drugs either amprolium or diclazuril detected lesser pathological changes not better

than in vaccinated birds. Since, an application of vaccination revealed apparently normal structure of the intestine.

Finally, **it could be concluded** that vaccination with live oocysts elicited a significant protection against coccidiosis (naturally acquired coccidial infection), while maintaining bird flock in a good performance similar to, if not better than, that obtained with conventional anticoccidial medication.

CONCLUSION

Infection with coccidian parasites leads to economic losses resulting from malabsorption of nutrients associated with decreased average body weight, feed intake, feed conversion rate, weekly body gain, and possibly increased mortality. Severe pathological changes varying from the local epithelial destruction and damage of the intestine to systemic deterioration and hemorrhages were also detected.

Vaccination using live oocysts offered a significant protection against naturally acquired coccidial infection, while maintaining bird flock in a good performance similar to, if not better than, that when compared with conventional anticoccidial medication.

It could be concluded that live oocyst vaccination is currently a realistic alternative and compensate to anticoccidial products for the prevention of coccidiosis in the broilers. It has been shown to be an effective tool for the generation of the immunity and protection against subsequent E. challenge, as evidenced by increased MBW gain.

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الملخص العربي

يعرف الكوكسيديا بأنه مرض طفيلي واسع النطاق ذات تأثير اقتصادي شديد على إنتاج وصناعة الدواجن. تؤدي العدوى بطفيليات الكوكسيديا إلى خسائر اقتصادية ناتجة عن سوء امتصاص العناصر الغذائية. أدى إلى تغييرات مرضية ملحوظة مع تدمير شديد للغشاء المخاطي في الأمعاء. تطبيق العقاقير المضادة للبكتيريا أو استخدامات التطعيم باستخدام البويضات الحية يمكن أن يوفر حماية كبيرة ضد الكوكسيديا.

ولقد أسست الدراسة الحالية على عدد مائتي من الدجاج الذي تم الحصول عليه من المفرخات التجارية. تم تقسيمهم بالتساوي إلى ٥ مجموعات تحتوي كل منها على ٤٠ فرخًا ؛ يتم تغذية جميع الكتاكيت على حصص عادية خالية من أي دواء مضاد للبكتيريا. يتم الاحتفاظ بجميع الفئات تحت نفس الظروف وتلقى نفس الإجراءات من برنامج الإدارة والتطعيم. وتم تصنيف الطيور إلى ٥ مجموعات (١ ، ٢ ، ٣ ، ٤ و ٥) على النحو التالي: المجموعة (١) ، تم استخدامها كمجموعة سلبية مراقبة (غير مصابة ولم يتم تحصينها ضد الكوكسيديا ولم تتلق أي أدوية مضادة للبكتيريا). المجموعة (٢) ، استخدمت كمجموعة إيجابية السيطرة (المصابة تجريبيا مع الايميريا ولكن لم يتم تطعيمها ضد الايميريا ، وليس تلقي أي أدوية مضادة للميكروبات). المجموعة (٣) ، تم تطعيمها ضد الايميريا باستخدام لقاح كوكسفاك - د داخل العين في اليوم الثاني من العمر. المجموعة (٤) ، تم الحصول عليها من عقار أمبروليوم كدواء مضاد للكوكسيديا (امبروكسين ٢٠ % شركة فارما سويد) ١٢٥ جم / ٢٠٠ لتر من مياه الشرب (١٢٥ جزء في المليون من أمبرليوم) لمدة ٧ أيام. المجموعة (٥) ، تم استلام ديكلازوريل كدواء مضاد للاكتئاب. أضف ٥٠ مل لكل ٢٠٠ لتر من مياه الشرب لمدة ٤٨ ساعة (داى كلاكوكس أفيكو).

بقيت جميع المجموعات تحت الملاحظة اليومية مع تسجيل اية اعراض أو وفيات ، وتم تجميع فضلات البراز لحساب البويضات في اليوم ٧، ١٤،٢١ و ٢٤ من العمر. كما تم تسجيل وزن الجسم ومعدل التغذية ومعدل تحويل التغذية لحساب معدل تحويل التغذية لجميع المجموعات. وعلاوة على ذلك ، تم تسجيل النتائج التشريحية المرضية والهستوباثولوجية.

أثبتت النتائج وجود أعراض اكلينكية ملحوظة بين المجموعة المصابة بإيميريا تينيلا ، وتمثلت الاعراض في ضعف الأداء والخمول وانخفاض وزن الجسم والريش المتساقط بالإضافة إلى الهزال. وأظهرت المجموعات الملقحة والمعالجة معدل أعلى في وزن وأداء الجسم مع نشاط جيد بالمقارنة مع المجموعة المصابة الغير معالجة.

انخفض معدل تحويل الغذاء والأرباح الأسبوعية بشكل ملحوظ بين مجموعة إيميريا فقط ويعزى ذلك إلى انخفاض استهلاك الخلاصة. أظهرت المجموعات الأخرى أما الضابطة الغير مصابة او المجموعات المعالجة تحسنًا في معدل تحويل الغذاء والمكاسب الأسبوعية. مع ارتفاع معدل الوفيات المسجلة بين المجموعة المصابة بالعدوى إيميريا. بينما ، اكتشفت مجموعات أخرى والتى تضم عقاقير واللقاح انخفاضًا في معدل الوفيات مقارنةً بمجموعة الضبط والتحكم.

كشف اعداد البويضة عددا أعلى في السيطرة على المجموعة الإيجابية المجموعة المصابة بالعدوى إيميريا فقط. بينما سجلت المجموعة التي تم تحصينها عددًا قليلًا في تعداد البويضة تليها مجموعة أمبرليوم ثم مجموعة ديكلازوريل.

أما بالنسبة للصفة التشريحية فقد أظهرت المجموعة الضابطة رؤية طبيعية للأمعاء ومظهر سليم ، وأوضحت المجموعة التي تم تلقيحها فقط بالإيميريا نزيف حاد مع محتويات معوية دموية ، وكانت مجموعة الأمبرولايوم والديكلازوريل تعانى من نزف خفيف. ومن الناحية الهستوباثولوجية ، فقد تسببت إيميريا تينيلا في حدوث تغيرات مرضية شديدة تتميز بنخر واسع وتدمير الأمعاء. واكتشف استخدام العقاقير المضادة للبكتيريا إما أمبرليوم أو ديكلازوريل تغييرات مرضية لكن أقل وليست أفضل من الطيور التى تم تطعيمها ضد الكوكسيديا. ولذلك يمكننا أن نثبت أن تطبيق التطعيم ضد الكوكسيديا أظهربنية طبيعية على ما يبدو للأمعاء.

أخيرًا ، يمكن أن نستنتج أن للتطعيم باستخدام البويضات الحية أثارا حماية كبيرة ضد الكوكسيديا (عدوى الكوكسيديا المكتسبة بشكل طبيعي) ، مع الحفاظ على قطيع الطيور في أداء جيد مماثل ، إن لم يكن أفضل من الذي تم الحصول عليه باستخدام الأدوية التقليدية المضادة للبكتيريا.

مقارنة بين بعض الادوية الوقائية والتحصين ضد

الكوكسيديا فى الدجاج

مقدمة من:

مصطفى عبدالقوى على

بكالوريوس العلوم الطبية البيطرية

كلية الطب البيطري – جامعة جنوب الوادى - ٢٠٠٩

للحصول على درجة الماجستير فى العلوم الطبية البيطرية

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٢٠٢٠م - ١٤٤١هـ