



## Highlighting on The role of Zinc Oxide and Silver Nanoparticles as An Effective Coccidiostats in Broilers

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**R**ECENT advances in nanomedicine have made it an attractive option for treating a range of diseases. Zinc oxide nanoparticles (ZnO NP) and silver nanoparticles (AgNP) were examined for their anticoccidial properties *in vivo*. This study aimed to highlight the effectiveness of zinc oxide and silver nanoparticles in preventing coccidiosis in broilers. In this study, *Eimeria* oocysts were synthesized, characterized, and analyzed histopathologically. TEM (transmission electron microscope) and SEM (scanning electron microscope) were used to characterize Zinc oxide nanoparticles enclosed in nanospheres of uniform spherical shape and size, while produced Ag nanoparticles were spherical. They showed high zeta potential, which indicates high colloidal stability. ZnO nanoparticles prepared by X-Ray diffraction analysis showed a high crystalline structure. Chemical homogeneity was evident from the XRD curves of AgNPs and ZnO NPs. The obtained size of Ag-NPs matched that of TEM, SEM and zeta potential values, demonstrating excellent stability in aqueous solutions. According to PCR tests, the prepared ZnO NPs and Ag-NPs have superior efficacy and potential compared to other anticoccidials. Zinc oxide nanoparticles showed potential anticoccidial efficacy compared to other groups. Results were confirmed by Histopathological examination.

**Keywords:** SEM (scanning electron microscope), TEM (transmission electron microscope), PCR, ZnO NPs, Ag-NPs

### Introduction

Coccidiosis is an enteric disease caused by infection with one or more species of *Eimeria* species that causes high economic losses in the poultry industry worldwide [1]. *Eimeria* species belonging to the phylum Apicomplexa, including *Eimeria maxima*, *Eimeria tenella*, and *E. acervulina* [2]. Each species of *Eimeria* differs in terms of the site of invasion, pathogenicity, and type of lesion. Diarrhea, weight loss, and mortality are the clinical manifestations of cecal

coccidiosis [3]. There are several species of *Eimeria.spp*. Each species has its own host and site, so any animal can host several of them [3]. Life cycles of *Eimeria* consist of two phases: sporogony, an exogenous phase, and schizogony, an endogenous phase. During the first phase of infection, gut cells are destroyed in large numbers by sporozoites, which are banana-shaped motile cells with complex structures that are capable of invading host cells [5].

Sporozoites are the result of protoplasm

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segmentation when the sporont that surrounded with ovoid cell wall that become immature oocyst which varies in size and shape according to the type of *Eimeria* species [6]. Life cycle of *Eimeria* initiated by ingested of sporulated oocyst by a susceptible host, and the sporozoites are released in the intestine from oocysts under the effect of digestive enzymes and mechanical disruption. Stress by carbon dioxide (CO<sub>2</sub>), which causes the rupture of the micropyle and increase in the permeability in the oocyst are the main mechanisms that lead to a collapse of the contents of the oocyst in a hypertonic salt solution [7]. Each species of *Eimeria* differs in the optimal concentration of CO<sub>2</sub>, time of incubation and the temperature at which the sporozoites liberated from oocyst. Trypsin has an important role in digesting the stiga body which causes a hole in the sporocystic membrane and it also activates the entire sporozoites to be liberated.

It also allows digestion enzymes to enter sporocysts through altering the micropyle or the lipoprotein of the stiga body, and it facilitates the release of sporozoites [8]. Amylopectin is used by the sporozoite to produce energy during excystation and invasion [9]. Cecal cells were infected with free sporozoites and developed inside a parasitophorous vacuole (PV) into a rounded and growing organism called the trophozoite, and then it becomes a meront during the first merogony generation.

A sporozoite's growth alters the endothelial cell, and its nucleus becomes larger. An enlarged nucleolus, scattered chromatin; Two concentric zones of cytoplasm rather than a vacuolated appearance. Its cells become hypertrophic in response to sporozoite growth. To accommodate meront development, the host cell nucleus migrates to the periphery from a random distribution. In *Eimeria* trophozoites, merogony occurs through multiple nucleus divisions without cytoplasm division, resulting in ellipsoidal structures called blastophores with a peripheral layer of nuclei. Every nucleus has a merozoite that grows radially around it. During the phase, merozoites are formed by cytoplasmic division. Upon maturity, the merozoites escape the intestinal lumen and travel to the large intestine, where they enter new cells. The second merozoites then invaded adjacent epithelial cells, causing sexual gamogony to take place. A few merozoites develop into male gamonts and the female macrogamete forms a large, mononuclear, spheroid cell. The formation

of gamonts caused the mucous membranes of the jejunum, ileum, and caecum to be destroyed, resulting in poor absorption and diarrhea [10]. A number of experiments in the field use nanoparticles as drug-carriers to deliver and transport medicinal drugs [11]. Nanoparticles can carry and deliver medicinal drugs in many ways. Due to the poor effectiveness of some medications, nanomaterials have gained considerable interest in biological and medical applications. In vivo and in vitro treatment strategies can be adapted to nanoparticles since their size and form are similar to biological molecules [12]. In many previous studies, nanoparticles have been used to improve poultry and animal productivity, but they also face the challenge of antibiotic resistance to microbes [13].

It has been found that adding zinc to poultry diets plays a vital role in growing bones, muscles, feathers, and skin, increasing zinc bioavailability, increasing weight gain, and improving food conversion efficiency. In addition, zinc is necessary for the activity of approximately 250 to 300 enzymes in the body and participates in several enzymatic functions and metabolic processes. In general, it improves immune responses by increasing IgY production and lymphocyte counts. Since Zn is toxic at high concentrations, it is preferable not to use high concentrations of ZNPs [14].

It has been shown that ZNPs reduce parasite development before inactive oocysts are formed, or possibly reduce the ability of ZNP to cross the gastrointestinal tract and spread further through the bloodstream and into target organs, leading to an increased immune response and resistance to infection [15].

Moreover, ZNPs have been shown to protect rabbit livers from coccidiosis infection. Recent studies have reported ZNPs as an effective alternative for treating coccidiosis. ZNPs have been shown to exhibit anti-neurological bilharziasis effects in mice infected with bilharziasis [16]. Coccidiosis can be reduced using zinc oxide nanoparticles, especially at 60mg/kg. Silver is an element that occurs naturally in the environment and is considered non-toxic, non-allergic, and not accumulated in the body. Compared to silver salts, metallic silver nanoparticles (below 200 nm) in solid or colloidal form exhibit a higher microbiological effect. Silver nanoparticles have been successfully used as an antimicrobial agent against *Chlamydomonas reinhardtii* in several

studies [17]. The silver level in broilers' edible parts such as breast, femurs, and livers is estimated to be approximately 0.1 mg/kg. In humans, this level is not toxic, but as nanoparticles, it may be toxic. It is unknown whether the silver in tissues is ionic or nanoparticles. [18]. Despite the fact that silver nano particles may kill coccidia in broiler intestines based on how many oocysts are excreted, administering silver nano particles to their drinking water did not affect their growth performance. The purpose of this study is to highlight the effectiveness of zinc oxide and silver nanoparticles as a coccidiostat in broilers.

### **Material and Methods**

#### *Collection of the parasite:*

To obtain the infective stage (sporulated oocyst), stool samples were placed in a 2.5% potassium dichromate solution for 5 days at AHRI (Animal Health Research Institute) in El - Giza Province. To obtain clear oocysts, saturated saline (saturated sodium chloride) was added to the flotation solution [19].

#### *Oocyst propagation:*

We used 121 day old broiler chicks raised in hygienic conditions (ration and water were free of anticoccidial drugs) in this research. A total of 100 chicks were inoculated with 28,500 sporulated oocysts suspended in 1ml of PBs for oocyst propagation [20].

#### *Counting of the sporulated oocyst:*

For infecting broilers with caecal coccidian, oocysts were calculated using a Mac-master. We counted all eggs inside each chamber, ignoring those outside of the squares, then multiplied the total by 50 to find the number of sporulated oocysts in every gram of faeces. Using a light microscope and measuring the sporulated oocyst using a micrometer [21], the parasite was diagnosed by a direct smear from the collected sporulated oocysts.

*Morphological identification of Eimeria tenella oocyst:* The sporulated oocysts were identified by micrometer according to [22].

*Molecular identification of Eimeria tenella isolate by PCR:* The sporulated oocysts were identified molecularly using PCR according to [23].

Characterization and preparation of ZNOPs & silver nanoparticles:

#### *The synthesis of zinc oxide nanoparticles*

Ultrasonic irradiation was used to synthesize

ZnO NPs. An example of such a procedure is using zinc nitrate ( $\text{Zn}(\text{NO}_3)_2$ , Loba Chemie, India) as a source material [24]. Using deionized water and 2 grams zinc nitrate, nanoparticles of zinc oxide were synthesized. An ultrasonic irradiation of 91% amplitude and a cycle of 0.73 was performed with a Hielscher UP400S (400 W, 60 kHz, Germany) on the dissolved zinc nitrate solution at 55°C for 25 minutes. After the zinc nitrate solution is formed, drop by drop the ammonia solution is added to it under ultrasonic irradiation.

#### *Silver nanoparticle synthesis*

Youssef et al. [25] previously described the synthesis of Ag-NPs at Egypt's national research institution. As precursor materials, silver nitrate and trisodium citrate (lab grade, Sigma, USA) were used. In order to synthesize silver colloid, 50 ml of 0.001 M  $\text{AgNO}_3$  were boiled, then 5 ml of 1% trisodium citrate solution were added drop by drop while vigorously stirring and heating until their color changed to pale yellow by following the following equation ( $4\text{Ag} + \text{C}_6\text{H}_5\text{O}_7\text{Na}_3 + 2\text{H}_2\text{O} \rightarrow 4\text{AgO} + \text{C}_6\text{H}_5\text{O}_7\text{H}_3 + 3\text{Na}^+ + \text{H}^+ + \text{O}_2\uparrow$ ). After removing the product from the heater, stirring, allowing it to cool to room temperature, and collecting it in dark bottles, the product was then collected in a heat resistant container.

#### *An investigation of the characteristics of nanoparticles prepared from silver and zinc oxides*

In this study, synthesized ZnO-NPs and Ag-NPS were analyzed using X-ray diffractometer (XRD, D8-Discover, USA) to determine the chemical structure and phase of crystal under condition of 1600W speed scan 0.05. ZnO-NPs and Ag-NPS were analyzed using scanning electron microscopes (SEMs, JSM-6701F Plus, JEOL, USA) and transmission electron microscopes (TEMs). In order to examine the samples by SEM, a gold grid was prepared for supporting the NPs, but first ZnO-NPs and Ag-NPS were diluted (1:10) with doubled deionized distilled water and sonicated for 30 minutes in an ultrasonic cleaner (Elma, Germany). The grids were then coated with ZnO-NPs and Ag-NPS and allowed to dry at 60°C before SEM analysis.

#### *Diagnosis of E. tenella oocysts*

To obtain sporulated oocysts, we placed the stool of infected broiler chickens in a 2.5% potassium dichromate solution for five days. To obtain clear oocysts, saturated saline (saturated sodium chloride) was added to the flotation solution.

In order to calculate how many oocysts were required to infect the broilers with caecal coccidia, a Mac-master was used. The number of sporulated oocysts per gram of feces was calculated by counting the eggs within the grid of each chamber, disregarding those outside the squares. Micrometer measurements of sporulated oocysts confirmed the diagnosis of the parasite by direct smear from collected sporulated oocysts.

#### *Experiment animals*

During the trial period, one-day-old broiler chicks were obtained from Cairo's poultry farm (Basatin) and were bred in clean boxes with bedding. The same environmental conditions were applied to all groups of broilers, including feeding and lighting as well as preventive health care.

#### *Infection of the animals:*

To check for the presence of *Eimeria* oocysts, stool samples were collected daily for seven days before infection. In this experiment, infective strains were detected by PCR. Six groups of 20 birds each were used for the experiment. 120 chicks are infected with 28,500 sporulated oocyst. The feces were examined after 10 days post infection using Mc-master technique. A control group (G1) was given distilled water as a negative control (without infection). A positive control of 101,400 mature sporulated oocysts was given to G2 (group that was infected) as a positive control. In G3, sporulated oocysts were dosed with 60 mg of zinc oxide, in G4 sporulated oocysts were dosed with silver nanoparticles (40 mg) before treatment, in G5 sporulated oocysts were dosed with diclazuril (1 ppm), and in G6, sporulated oocysts were dosed with Amprolium (150 ppm) before treatment. Fecal samples were examined using Mc-master technique after treatment with previous drugs.

#### **Statistical analysis**

Data were analyzed using IBM SPSS statistics 20 software using one-way analysis of variance (ANOVA) followed by the Duncan multiple comparisons test for post hoc analysis. A  $p < 0.05$  was considered statistically significant. Non-significant when the P value was  $> 0.05$ . Values were expressed as mean  $\pm$  SD.

#### *Ethical approval*

The study protocol was approved by The Institutional Animal Care and Use Committee (Vet. CU. IACUC) of the Faculty of Veterinary

Medicine, Cairo University, Egypt with code "Vet CU 08072023664".

## **Results**

#### *ZnO nanoparticles characterization XRD*

**Figure 1.** Synthesized ZnO NPs XRD curve. As seen from the XRD pattern, the prepared ZnO NPs had a high diffraction peak intensity, indicating a high crystalline structure. According to the Brucker database COD no. 2300113, ZnO NPs have dominated diffraction peaks in XRD curves. There are, however, diffraction peaks associated with (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202). Moreover, oxygen represents 20.8% of the sample, while zinc represents 79.2%. As shown in Figures 2 and 3, ZnO NPs are encapsulated in nanospheres of uniform spherical shape and size.

#### *Dynamic light scattering (DLS)*

Using the dynamic light scattering (DLS) method, ZnO NPs were examined for their size distribution and exposed to possible zeta estimates. ZnO NPs had a homogeneous size distribution and a mean size of 35 nm, which was compatible with the mean size observed in TEM and SEM images. A - 32.02 mV zeta's advantage was found for ZnO NPs. Accordingly, the high zeta potential leads to higher colloidal stability in water, which, in turn, can be attributed to ZnO NPs' high bioactivity.

#### *Characterization of the prepared silver nanoparticles XRD*

AgNPs exhibited the best amorphous state (zero peaks) without any additional peaks, indicating homogeneity.

#### *SEM and TEM for Ag-NPs*

Figure 4 shows (A) TEM 2D and (B) SEM 3D images of the Ag-NPs. SEM images were used to confirm the spherical structure of Ag-NPs produced in nano-sized form, followed by TEM images. Figure 5 shows SEM (A) and TEM (B) images that prove the nanocomposite was created.

#### *Zeta size and zeta potential*

In order to determine the size and stability of Ag-NPs in aqueous media, the zeta size and potential of the produced Ag-NPs were measured. A 25 nm particle size and 35 mV zeta potential are obtained for the produced Ag-NP. It was confirmed that these particles are extremely stable in aqueous conditions by TEM, SEM, and zeta potential measurements.



*Diagnosis of Eimeria tenella:*

*Eimeria tenella* stages was diagnosed morphologically using light microscope. Then the diagnosis confirmed using PCR Fig(e).

**Based on the results of the experiment, G1 showed zero Eimeria tenella oocysts.** G2 showed 28,500 *Eimeria tenella* oocysts, while G3 showed 14,200 *Eimeria tenella* oocysts after being treated with 60mg zinc oxide. 60,450 *Eimeria tenella* oocysts were found in G4, the infected group treated with silver nanoparticles of 40 mg. Diclazuril-treated group G5 showed 8,200 *Eimeria tenella* oocysts, while amprolium-treated group G6 had 4,486 oocysts. Fig. (8)

*Histopathological results*

Histopathological examination results illustrated in fig. (1) to fig. (12).

*Coccidiosis score lesions*

Coccidial lesion score were illustrated and demonstrated in figs. (14&15)

Fig 14. Coccidial lesion score in different groups

C: control.

N: number of birds.

0: No coccidiosis.

1: Mild infestation.

2: Moderate infestation.

4: Severe infestation.

Negative control group showed apparently normal structures in all examined birds. Positive control group showed massive infiltration with all eimeria developmental stages (gametocytes, second generation of schizonts contain sheaves of merozoites, and oocysts) that invade the lining epithelium of cecum. Degeneration and necrosis of lining epithelium were observed with sloughed epithelium and hemorrhage in the lumen. The mucosal glands were heavily infiltrated with micro and macrogametocytes and oocysts. The lamina propria exhibited edema and hemorrhage. Silver group: One bird out of two has no coccidial infestation, while the other two has moderate infestation, in addition to hyperplasia of lining epithelium with mild degeneration and activation of goblet cells. Compressed and shrinkage in many stages of *Eimeria* were observed leaving large hollows. Amprol group: apparently normal to mild infestation was noticed, associated with several hollows in the lining epithelium with no

and/ or compressed macrogametes and oocysts, as a result of treatment. Dicloazuril group showed very mild infestation in one bird and the other two showed no lesions. Zinc group showed no coccidial infestation.

**Discussion**

It was our aim to demonstrate the effectiveness of zinc oxide and silver nanoparticles as coccidiostats in broilers through this study. A number of oocysts were synthesized, characterized, and tested with PCR and histopathological confirmation. Results from SEM and TEM revealed ZnO nanoparticles encased in spherical nanospheres of uniform size and shape, while Ag nanoparticles exhibited spherical structures. Water has a higher colloidal stability due to its high zeta potential. The ZnO NPs were found to have a high crystalline structure based on XRD results. ZnO nanoparticles have characteristic diffraction peaks. XRD curves for AgNPs showed that they were amorphous (zero peaks) because the chemical composition was homogenous. According to the TEM, SEM, and zeta potential values, the size of Ag-NPs obtained was consistent with their TEM, SEM, and zeta potential sizes. After the diagnosis was confirmed with PCR, the prepared ZnO NPs were found to demonstrate superior efficacy ( $p < 0.05$ ) compared to the other groups. When comparing the coccidial lesion scores of the different groups, ZnO nanoparticles performed better than silver nanoparticles ( $p < 0.05$ ).

In accordance with our findings on ZnO NPs, Hatab et al., [26] found that inclusion levels of biogenic synthesized ZNO NPs at 40 or 60 mg/kg diets significantly improved blood indices, physiological changes, antioxidant status, immunological responses, intestinal microbial population, and finally broiler chick health. A dose of 40 mg/kg of Zn delivered as nanoparticles to broiler diets proved to be more effective than high doses and was safe for all broilers.

Using zinc oxide nanoparticles (ZNPs), Lail et al. [27] studied their anticoccidial properties *in vivo*. Upon characterization, ZNPs were revealed to be crystalline in nature, with a smooth and spherical surface as well as a diameter ranging between 10 and 15 nm. ZNPs were assessed for their morphology and size using field emission scanning electron microscopy, and their crystalline properties were determined using X-ray diffraction. *Eimeria papillata* infected mice produced  $29.7 \times 103 \pm 1,500$  oocysts/g feces on

day 5 postinfection. This output was significantly decreased, to  $12.5 \times 103 \pm 1,000$  oocysts, in mice treated with ZNPs. The jejunum was also inflamed and injured by infection. A decrease in glutathione levels and goblet cell numbers were observed in mice jejunum as a result of the increased production of nitric oxide and malondialdehyde and an increase in inflammatory histological scores. Treatment with ZNPs significantly altered all of these infection-induced parameters. As a result, our results suggest that ZNPs provide protection against *E. papillata*-induced coccidiosis.

*Zinc oxide nanoparticles (ZnONPs) were synthesized from Nigella sativa using biosynthetic methods and evaluated on Eimeria tenella infected broilers by Anah et al., [28].* ZnONPs with spherical shapes and a Zeta potential of 30 mV were found by scanning electron microscopy. A significant ( $p < 0.05$ ) decrease in oocyst shedding and anti-coccidial index was observed in infected birds treated with ZnONPs. It was found that ZnONPs treated animals showed a significant decrease in the level of aspartate transferase and alanine transferase, while they had a significantly higher level of antioxidants like catalase and superoxide dismutase. Pro-inflammatory cytokines like IL-2 and TNF- $\alpha$  were significantly decreased by ZnONPs ( $p < 0.05$ ). The improved biogenic ZnONPs derived from *Nigella sativa* may have enhanced anticoccidial, antioxidant, and anti-inflammatory effects.

*Researchers tested zinc oxide nanoparticles (ZNOPs) at concentrations of 20, 40, and 60 mg/kg for their antimicrobial effects on Eimeria tenella oocysts in Anah et al., [28].* The oocysts of *E. tenella* were initially detected by compound optical microscopy in the feces of broilers at a veterinary hospital in Diwaniyah Province. During the first week of infection, ZNOP concentrations of 20 and 40 mg/kg exhibited varying levels of activity against *E. tenella*, while 60 mg/kg was the most effective in reducing excreted oocysts compared to the positive control and amprolium group, along with the appearance of mild symptoms and a mortality rate of 0.8%. Second week results showed a decrease in excreted oocysts and mortality. The birds recovered from infection after two weeks of treatment with 60 mg/kg ZNOP, showing a significant reduction in excreted oocysts. It appears that ZNOPs can combat *E. tenella* when anticoccidial agents are ineffective.

*AgNPs were shown to be effective in treating mice infected with Eimeria papillata by [29].* By treating infected mice with AgNPs, the jejunal mucosa improved, goblet cells increased, and meronts, gamonts, and oocysts developed less in the jejunum. As a result of AgNPs, feces also contained fewer oocysts. Researchers found that AgNPs had an anticoccidial effect on the jejunum of *E. papillata*-infected mice, suggesting that they could be used to treat the disease.

The anti-inflammatory and antioxidant effects of AgNPs can be attributed to this action. Histopathology of the jejunum of *E. pusillata*-infected mice revealed the presence of many lesions, vacuolations of the epithelium, and destruction of certain villi. Mice treated with biosynthesized AgNPs showed significant improvement in their jejunum after treatment with AgNPs. A similar improvement of histopathological changes was reported by [30] and [31] in the jejuna of *E. papulata*-infected mice after selenium nanoparticle treatment. An immune-defensive barrier is formed by the mucus secreted by the goblet cells in the intestine during food ingestion [32]. A number of studies have reported that *Eimeria* infection greatly affects goblet cells. In this study, histological sections of jejunum from *E. papillata*-infected mice showed reduced numbers of goblet cells on day 5 postinfection.

As a result, the present study indicated immature oocysts were found in the feces of the infected groups after one week of infection. The immature oocysts were kept in potassium dichromate and developed into mature sporulated oocysts after five days at 28°C in incubators, which was consistent with [33].

The sporulated *Eimeria tenella* oocyst measured was ovoid in shape, with a shape index of 1.2. The average length and width of the oocyst were  $(20.86 \pm 2.79)$ , and this result was about in agreement with [34] who reported that the shape index of the collected *Eimeria tenella* was (1.16). According to [35], the length of the ITS-1 region of the *Eimeria tenella* genome in the present study was 278bp. remodeling phase, despite the presence of fine interlacing collagen fibers [42].

## Conclusion

Nanomedicine can now treat a variety of diseases as a result of recent advancements. Silver nanoparticles (AgNP) and zinc oxide nanoparticles (ZnO NP) were investigated in vivo for their anticoccidial properties. In this study,

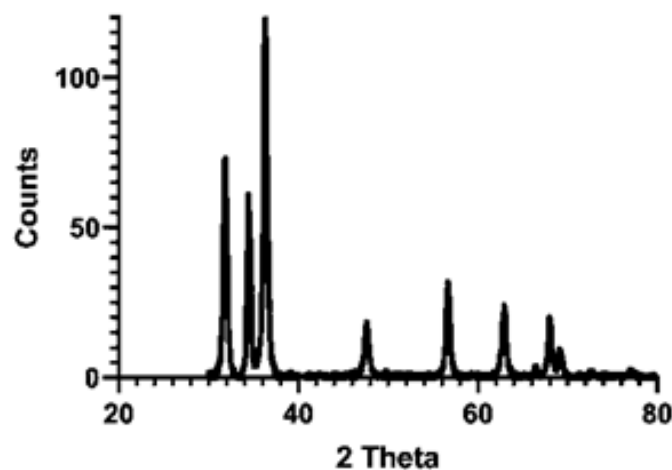
zinc oxide and silver nanoparticles were tested for their effectiveness in preventing coccidiosis in broilers. In this study, oocysts were synthesized, characterized, and analyzed histopathologically. TEM (transmission electron microscope) and SEM (scanning electron microscope) were used to characterize ZnO nanoparticles in nanospheres. ZnO nanoparticles prepared by XRD showed a high crystalline structure. XRD curves showed chemical homogeneity in both AgNPs and ZnO NPs. Zeta potential, TEM, and SEM measurements of Ag-NPs in aqueous solutions were consistent. According to PCR tests, ZnO NPs and Ag-NPs are superior to other anticoccidials in terms of efficacy and potential. Compared to silver nanoparticles, zinc oxide nanoparticles had a greater anticoccidial efficacy. Efficacy and potential were demonstrated in histopathological studies by zinc oxide nanoparticles.

#### *Conflict of interest*

There are no declared conflicts of interest. The corresponding author is ready to provide any detailed data upon request.

#### **Acknowledgement**

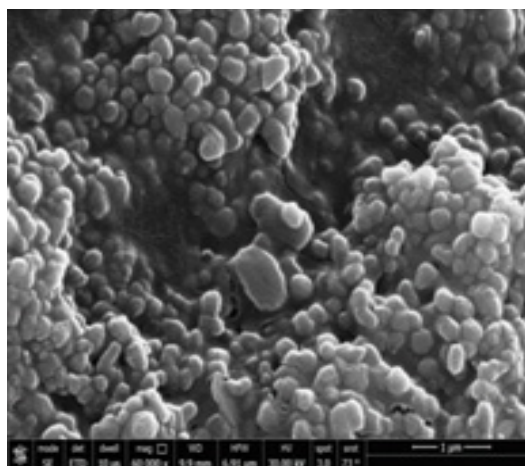
We would like to acknowledge faculty of Veterinary Medicine, Cairo University and doctor Sameh Hamid Ismail for his potential and valuable recommendations and effort.



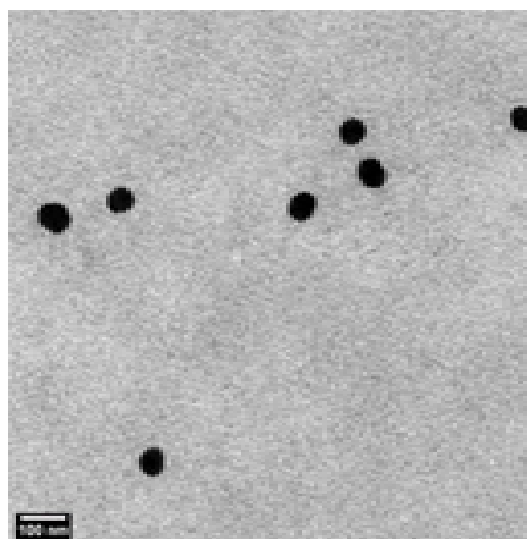
**Fig.1.** ZnO NP fingerprints according to COD no. 2300113 in the Brucker database.

**SEM & TEM**

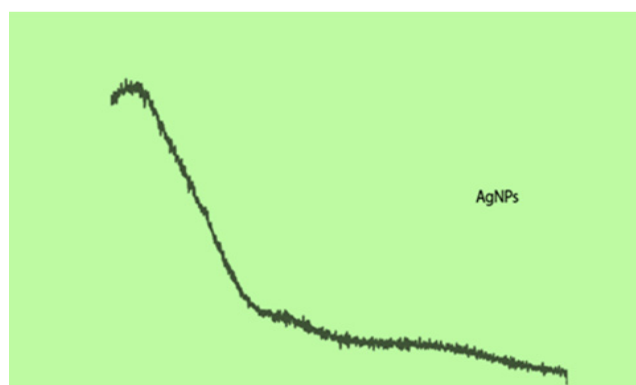
TEM and SEM results confirmed that ZnO nanoparticles are encased in uniform spherical nanospheres (Fig.2,3).



**Fig. 2.** Here is an image of the spherical monodispersed ZnO-NPs obtained by SEM.

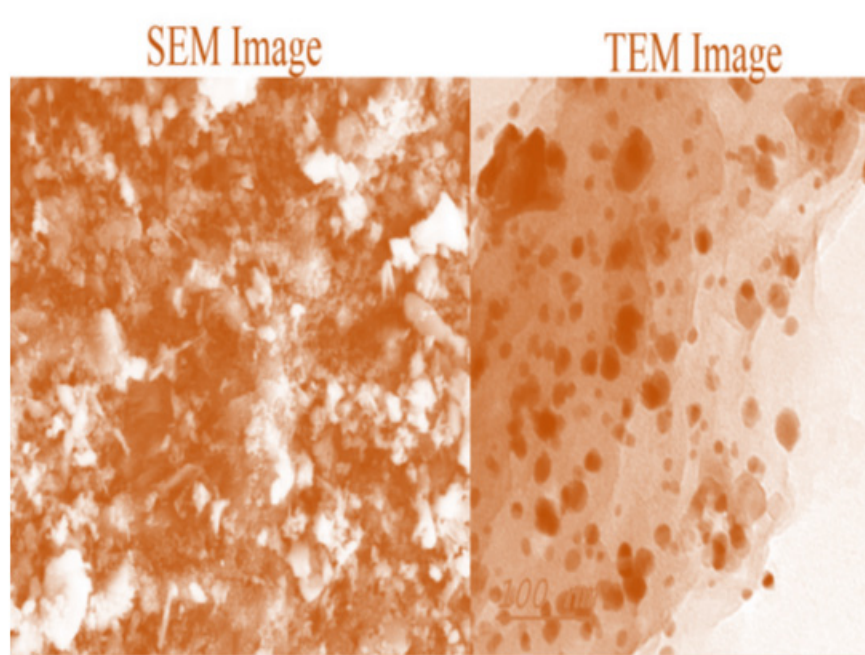


**Fig. 3.** ZnO-NPs nanospheres are illustrated in the TEM image.



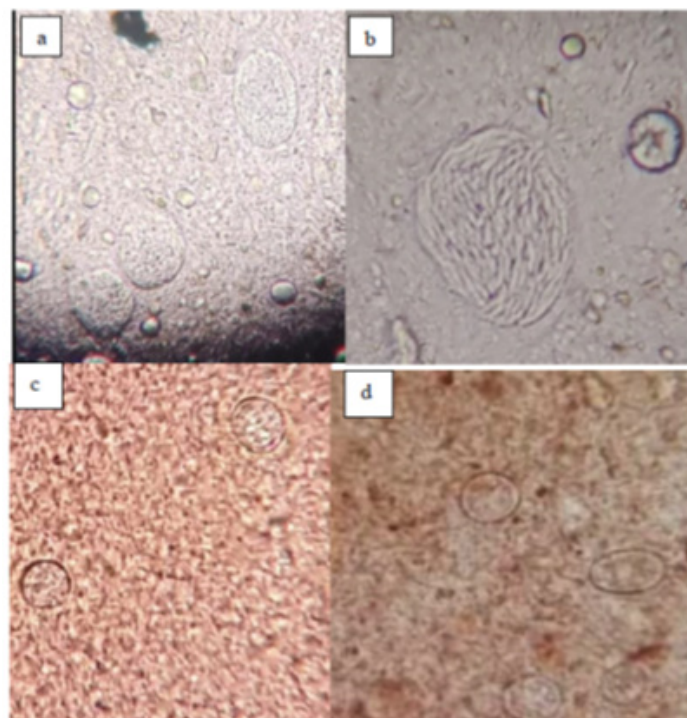
**Fig. 4.** XRD chart silver nanoparticles.





**Fig. 5. Silver Nanoparticles (Ag-NPs) SEM (A) and TEM images (B).**

**Eimeria tenella** stages was diagnosed morphologically using light microscope



**Fig. 6. Fig. a. First stage Eimeria tenella schizont in stool samples from infected chicks. Fig. b. shows Eimeria tenella schizonts in stool samples from infected chicks. Fig. c. Gametocytes of Eimeria tenella found in stool samples from infected chicks. Fig. d. An oocyst of Eimeria tenella is shown in a stool sample taken from an infected chick (40X).**

**Diagnosis of Eimeria tenella:**

*Eimeria tenella* stages was diagnosed morphologically using light microscope  
Then the diagnosis confirmed using PCR Fig(7)

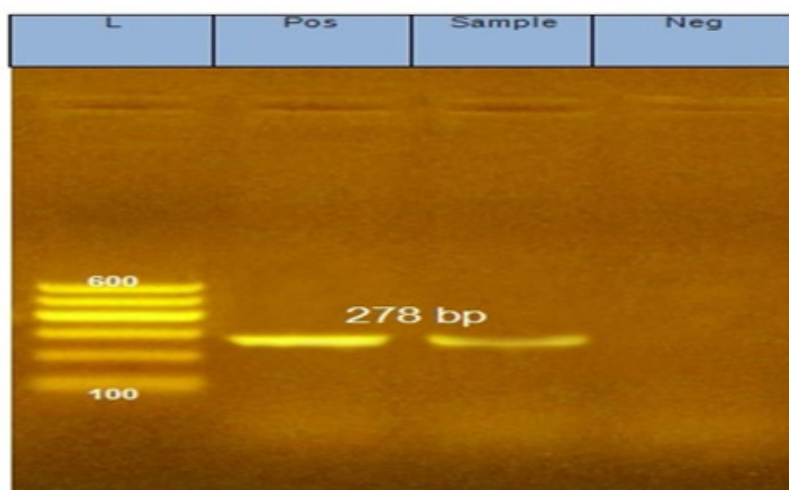


Fig.7. A representative gel picture showing the PCR amplification products from one isolate (*Eimeria tenella*) is provided in ITS-1 region was 278bp .

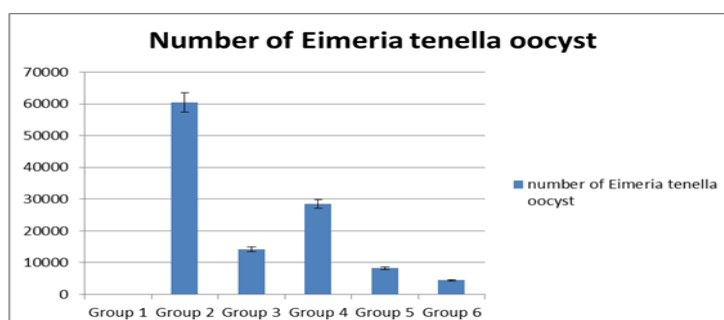


Fig. 8. Oocyst no. in each group.

Fig. 8. Oocyst no. in each group.

**Histopathological results**

Histopathological examination results illustrated in fig (9) to fig (20)

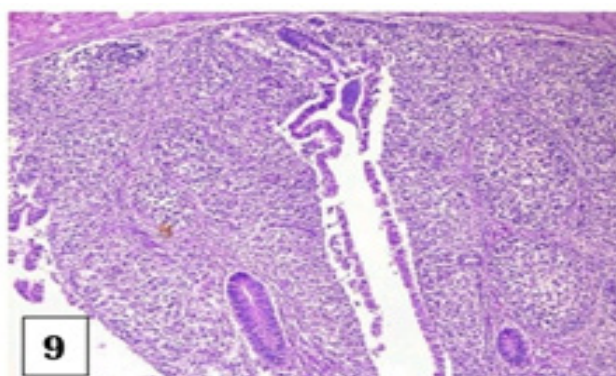
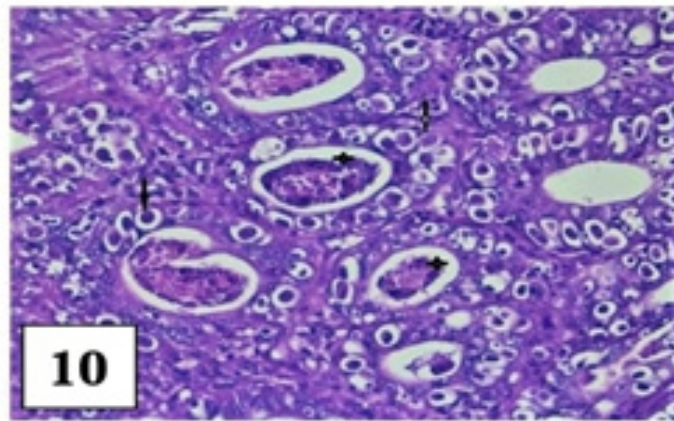
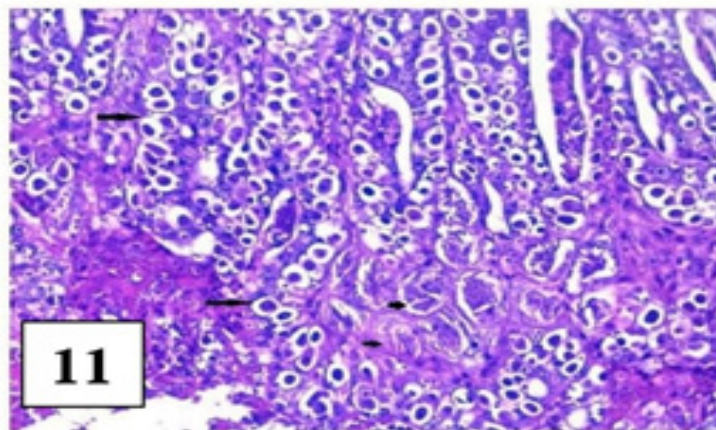


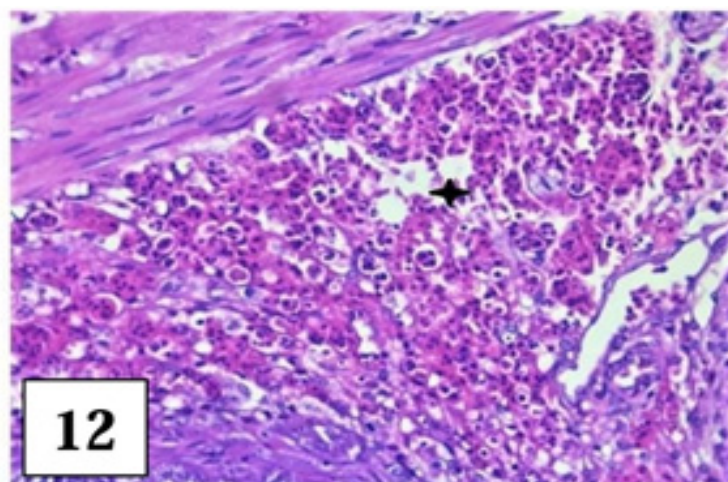
Fig. 9.Cecal tonsils of negative control group showing apparently normal architectures (H&E X100).



**Fig. 10.** Cecal tonsils of positive Control group showing severe infestation with macrogametes, microgametes (long arrows), shizonts containing merozoites (short arrows) and oocysts embedded in the lining epithelium

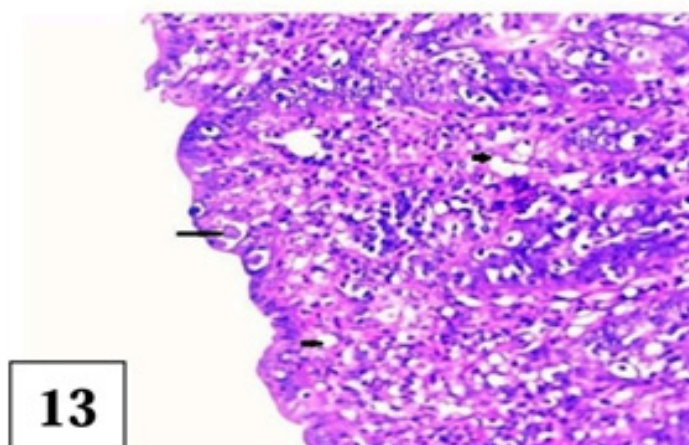


**Fig. 11.** Cecal tonsils of positive Control group showing severe infestation with macrogametes, microgametes, and oocysts embedded in the lining epithelium of mucosal glands (arrows) in lamina propria, in addition to eosinophilic necrotic debris (star) fill the lumen (H&E X400)

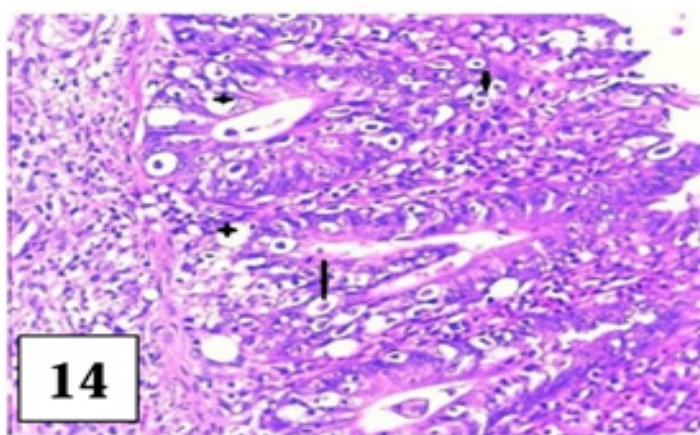


**Fig. 12.** Cecal tonsils of positive Control group showing severe hemorrhage and inflammatory cells infiltration in lamina propria (H&E X400).

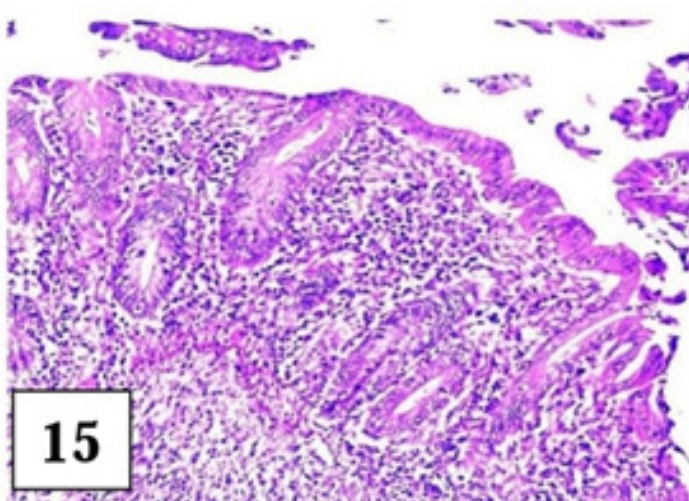




**Fig. 13.** Cecal tonsils of Silver treated group showing few developmental stages infest the lining epithelium (H&E X 400).



**Fig. 14.** Cecal tonsils of Silver treated group showing few compressed developmental stages (arrows) infest the lining epithelium of mucosal glands with several hollows (stars) (H&E X400).



**Fig. 15.** Cecal tonsils of zinc treated group showing no coccidia in the lining epithelium with regeneration in the epithelium (stars) (H&E X400).

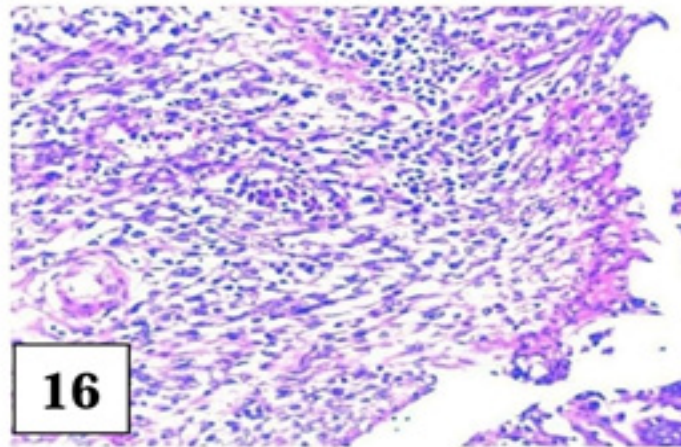


Fig. 16. Cecal tonsils of zinc treated group showing few focal aggregation of granulocytes. (H&E X400).

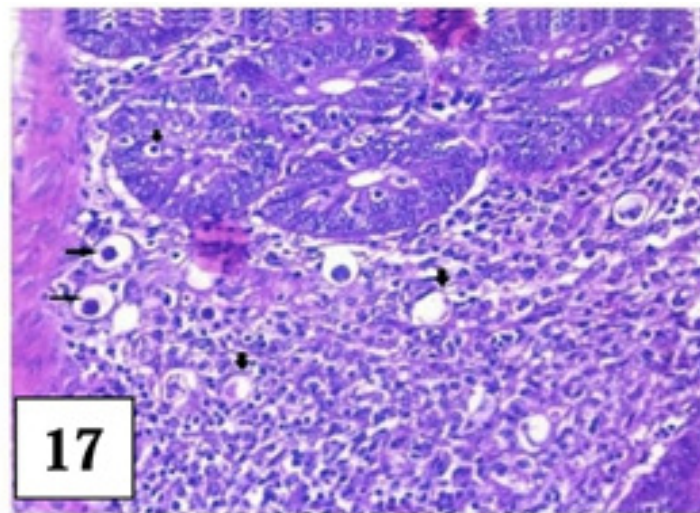


Fig. 17. Cecal tonsils of Amprolium treated group showing compressed macrogametes (arrows) and several hollows containing remnants of coccidia developmental stages short (arrows) (H&E X400).

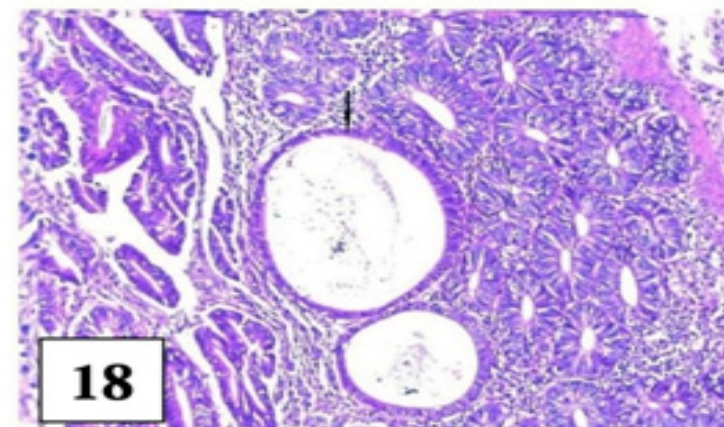
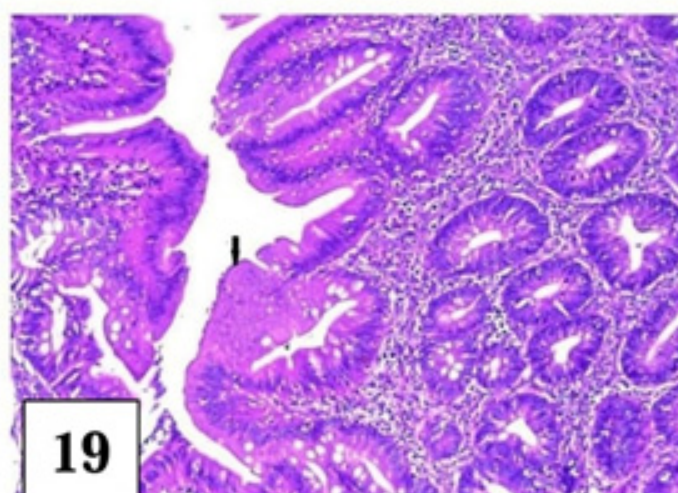
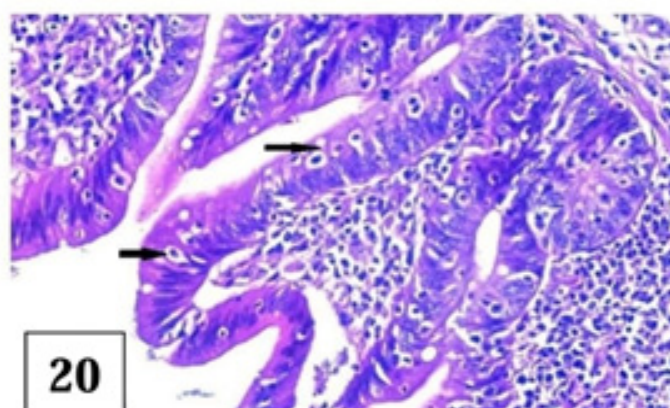


Fig. 18. The submucosa of Cecal tonsils of Amprolium treated group showing large cysts of mucosal glands (arrow), with very few compressed oocysts in other glands (circle) (H&E X200).





**Fig. 19.** Cecal tonsils of Diclazuril treated group showing regeneration in the lining epithelium (arrow) (H&E X 400).



**Fig. 20.** Cecal tonsils of Diclazuril treated group showing few compressed developmental stages of coccidia in the lining epithelium (arrow) (H&E X 400).

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## تسليط الضوء على دور أكسيد الزنك وجزيئات الفضة النانوية كمضاد فعال للكوكسيديا في دواجن التسمين

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جعلت التطورات الحديثة في الطب النانوي منه خيارًا جذابًا لعلاج مجموعة من الأمراض. تم فحص جسيمات أكسيد الزنك النانوية (ZnO NP) وجسيمات الفضة النانوية (AgNP) لمعرفة خصائصها المضادة للكوكسيديا في الجسم الحي. هدفت هذه الدراسة إلى تسليط الضوء على فعالية أكسيد الزنك وجزيئات الفضة النانوية في الوقاية من مرض الكوكسيديا في فروج اللحم. في هذه الدراسة تم تصنيع بويضات الإيميريا وتوصيفها وتحليلها نسجياً. تم استخدام TEM (المجهر الإلكتروني النافذ) و SEM (المجهر الإلكتروني الماسح) لتوصيف جسيمات أكسيد الزنك النانوية المحصورة في كرات نانوية ذات شكل وحجم كروي موحد، في حين كانت جسيمات Ag النانوية المنتجة كروية. يحتوي الماء على إمكانات زيتا عالية، مما يدل على الاستقرار العالي للمركب. أظهرت جسيمات أكسيد الزنك النانوية المحصورة بتحليل حيود الأشعة السينية بنية بلورية عالية. كان التجانس الكيميائي واضحًا من منحنيات XRD لـ ZnO NPs و AgNPs. يتطابق الحجم الذي تم الحصول عليه من Ag-NPs مع القيم المحتملة لـ TEM و SEM و zeta، مما يدل على ثبات ممتاز في المحاليل المائية. وفقا لاختبارات PCR، فإن ZnO NPs و Ag-NPs المحصورة تتمتع بفعالية وإمكانات فائقة مقارنة بمضادات الكوكسيديا الأخرى. أظهرت الجسيمات النانوية لأكسيد الزنك فعالية محتملة ضد الكوكسيديا مقارنة بالمجموعات الأخرى. تم تأكيد النتائج عن طريق الفحص النسيجي.

**الكلمات الدالة:** SEM (المجهر الإلكتروني الماسح)، TEM (المجهر الإلكتروني النافذ)، PCR، جزيئات أكسيد الزنك النانوية، جزيئات الفضة النانوية، التشريح المرضي.