

## Research Article



# Biology, Histopathology and Treatment Evaluation Against *Cryptosporidium meleagridis* on Infected Quails

KHITAM J. YAHYA\*, MOHAMMED T. S. AL-ZUBAIDI

Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

**Abstract** | *Cryptosporidium* is a ubiquitous protozoan parasite causing gastrointestinal disorders in various hosts worldwide. The current study was undertaken to study the biology of *Cryptosporidium meleagridis* and examine the anti-Cryptosporidial efficacy of curcumin in experimentally infected quails compared with that of paromomycin. This study carries out from September 2022 to January 2023 in Baghdad city, Iraq. Oocysts were isolated from naturally infected quails identified as *Cryptosporidium meleagridis* and used as a strain in experimental infection, about fifty birds were divided into five groups (G1: control negative group (con-), G2: control positive group (con+), G3: infected and treated with paromomycin, G4: infected and treated with curcumin and G5: uninfected and received curcumin). The oocyst shedding was recorded daily for 30 days. At 3, 8, 14, and 23 days post-infection (DPI), two quails from each group were killed humanely; ileum tissue samples were collected before and processed for histopathological evaluation. Improvement of tissue and the number of oocysts on the villi decreased after treatment. In comparison with G2, oocyst shedding was stopped at the end of the treatment period in both G3 and G4 without recurrence 10 days after drug withdrawal. The pre-patient periods (p.p) was 8-9 DPI. The maximum intensity was 11,000 oocysts per gram of feces. The first clinical sign was detected on 8<sup>th</sup> DPI. Top of shedding occurred on 14 DPI, The patient period was 16 DPI. No clinical signs appeared on experimentally infected birds, (incubation period of 8 days). Histopathology examination of the infected ileum showed the development stages of *C. meleagridis*. There were significant differences between the curcumin (4.33 mg/kg/day) and paromomycin (100 mg/kg/day) treatments. Curcumin was able to treat the infection and needed further evaluation.

**Keywords** | *Cryptosporidium*, Biology study, Histopathology examination, Curcumin, Paromomycin, Herbal medicine, Quails

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\*Correspondence | Khitam J. Yahya, Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; Email: kitam.jassim@gmail.com

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## INTRODUCTION

*Cryptosporidium* a genus of Apicomplexan parasitic protozoa is well known as a global protozoan parasite in both humans and a wide range of animals. In domesticated animals, Cryptosporidiosis may lead to severe economic loss due to mortality, weight loss, and medication cost. Quails that are infected with coccidian causes more pathology changes leading to severe bloody intestines and economic loss

(Al-Zarkoushi and Al-Zubaidi, 2021a). Oocysts that have sporulated and shed in the feces are instantly contagious. They are typically spread by the fecal-oral route, either directly or on food or water that has been contaminated even filtered water (Hadi, 2014). *Cryptosporidium* oocysts are destroyed by desiccation but can survive for over a year if kept in a cool, wet environment and for at least six months if kept at 4-6 °C (Gerace et al., 2019). Some birds may eventually pass away from the infection if it becomes

persistent and all three *Cryptosporidium* spp. were isolated from birds in Iraq (Faraj, 2014). There is currently no drug that can cure Cryptosporidiosis, so efforts are still needed to develop an effective drug and a wide variety of medications and components have been tested as a potential treatment for the illness (Shahiduzzaman and Dausgies, 2012).

One of the tiniest poultry species, the quail (*Coturnix Coturnix*), offers more benefits than chickens, including high production rates, lower feed consumption, and resistance to a variety of poultry diseases. They are also distinguished by their initial low expenses, which do not require a large area for farming, profitability, and amusement, therefore it represented a contemporary trend in the poultry sector (Hassan et al., 2020).

Curcumin (*Curcuma longa*) is a plant extract and is a very safe substance; there is no toxicity even at high doses, and it has anti-proliferative, anti-microbial, anti-inflammatory, antioxidant properties, and anti-protozoal actions against (*Plasmodium*, *Leishmania*, *Trypanosoma*, *Schistosoma*, *Babesia*, *Giardia*, and *Coccidia*). Our research is an experiment to discover a parasite cure. Numerous drugs and ingredients have been investigated as potential treatments for the condition, but there is presently no pharmaceutical that can cure Cryptosporidiosis, thus further work is required to create a potent therapy.

## MATERIALS AND METHODS

### STUDY AREA

About 100 birds obtain from Baghdad city, Iraq were examined to look for *Cryptosporidium* oocysts, positive samples were used for the preparation of infective doses.

### COLLECTION AND PURIFICATION OF OOCYSTS

The oocysts samples were obtained from infected quails from Baghdad city, Iraq. The direct examination was used to identify the infection, and the modified Kenyon's acid-fast approach was used to confirm it (Ma and Soave, 1983). The fecal suspension was sieved to remove debris and then washed three times with tap water to isolate the oocysts (2000 rpm, 10 min). The oocysts were then separated and purified using the discontinuous sucrose technique (Arrwood and Sterling, 1987). In a nutshell, Sheathers solution was made by mixing oocysts in 2.5 % aqueous potassium dichromate ( $K_2Cr_2O_7$ ) at 4 °C until the experiment (Rekha et al., 2016).

### ANIMAL GROUPS AND EXPERIMENTAL INFECTION

Quails of 2-3 days old were purchased from a nearby market in Baghdad city, their weight and feces were checked daily for five days. Ten quails were housed in a single cage, which had a temperature setting of 25 to 28°C and provided with food.

## EXPERIMENTAL DESIGN

Fifty birds without take care about sex were divided into five experimental groups, each consist of ten quails, with an average age of (7-8 days) and a weight of (14-17g).

G1: control negative group (con-) received distilled water, G2: control positive infected with  $10^3$  oocysts, G3: infected with  $10^3$  and treated by paromomycin, G4: infected with  $10^3$  oocysts and treated with curcumin, and G5: uninfected received curcumin only.

### PREPARATION OF TREATMENT DOSE

Curcumin: The recommended therapeutic dose of curcumin (*C. longa* C1386, Sigma- Aldrich, USA) is 4.33 mg/kg/day was suspended into 50µl of corn oil, as described previously (Cervantes-Valencia et al., 2018).

Paromomycin: The recommended therapeutic dose of Paromomycin (paromomycin sulfate, Sigms-Aldrich, USA) is 100 mg/kg/day was suspended into 100µl of distal water, as described previously (Cervantes-Valencia et al., 2018).

Numerous parameters were evaluated in groups of quails, including; the presence of clinical symptoms of clearance in infected quail; recording the pre-patent period and patint period, droop examination by hemocytometer chamber, determining the top of shedding, measured incubation period, and measuring the impact of drugs.

The drugs impact use in this study was measured according to (Xiao et al., 1999).

$$\text{Impact of drug} = \frac{\text{oocysts rate per 1 g of con.group} - \text{oocysts rate per 1 g of treatment group}}{\text{oocysts rate per 1 g of con.group}} \times 100$$

### OOCYST SHEDDING

Up to the final of the experiment, 0.5 g of fecal samples were taken daily directly from each quail in the several experiment groups to track oocysts shedding throughout the trial Fecal samples were examined directly under a microscope looking for oocysts and confirmed by a modified Zhen Neelsen stain. Oocysts number was counted every day by using a hemocytometer (×1000) (Ortolani, 2000).

### HISTOPATHOLOGICAL ANALYSIS

At 3, 8, 14, and 23 of treatment days, two quails from each group were slaughtered. This tissue sample was transported to the pathology and poultry diseases laboratory of veterinary medicine at Baghdad University and fixed individually in 10% neutral buffered formalin before being embedded in paraffin after the ileum was removed. Serial 5µm thick sections of ileum tissue blocks were cut out, and they were then stained with hematoxylin and eosin (H & E). The tissue sections were then put on a different

slide and inspected under a compound microscope with a 100x (Cardiff et al., 2014). Histopathological lesions were assessed as normal without alterations and without oocysts, mild with few alterations and few oocysts, and severe with severe damage and a high number of oocysts on villi.

### STATISTICAL ANALYSIS

The statistical analysis system, SAS Program (2018) was used to detect the effect of different factors on study parameters. The Chi-Square test was used to significantly compare percentages (0.05 and 0.01 probability) in this study. In order to study the variance in oocysts shedding among the different groups Kruskal-Wallis was used and the data obtained were shown as mean  $\pm$  standard error of mean (SEM).

## RESULTS AND DISCUSSION

### OOCYSTS SHEDDING

All infected and uninfected quails were normal throughout the experiment. There were no dead birds. The disease incidence of quails at 7 days of age was inoculated with  $1 \times 10^3$  oocysts/ quail was 100% without death. Macroscopic observation revealed no notable modifications. No animals perished throughout the experiment, as well.

Clinical symptoms, including foamy feces, dullness, and reduced weight gain, while post mortem showed a small intestinal distention with gas and mucus, start to manifest on the eighth day after infection (incubation period). At 8 to 9 days after infection, oocysts were initially noticed in quails droppings (pre-patent period) and maximum oocysts shedding occurred at 14 DPI, experimentally infected birds typically remained asymptomatic or had moderate symptoms. Before the experiment began, the weights of all quail ranged from (17-19) g at 7 days of age. During the infection, exactly after the fifth day of infection, weight gain in the infected group G2 was significantly reduced from (17-19) g to (14-16) g, whereas the weight of both treatment groups was increased to (25-30) g and (70- 75) g in G3, G4, respectively. It differs considerably from the control groups G1 and G5, which weigh respectively (95 – 100) and (98- 120) g.

### ANTICRYPTOSIDIAL EFFECT OF PAROMOMYCIN AND CURCUMIN

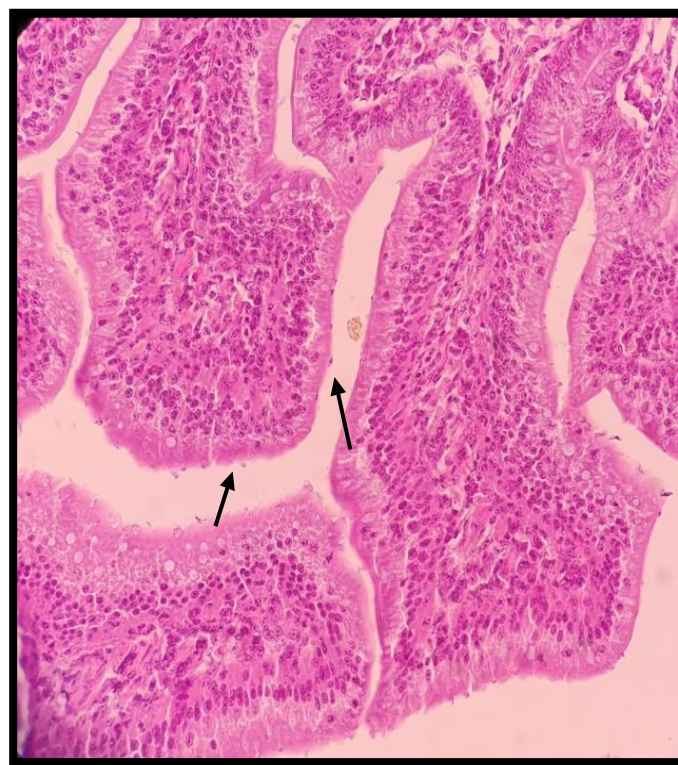
When treatment began on the 8 (DPT), the number of oocysts in one gram of drops was (2000, 2000, and 2250) oocysts per gram (OPG) in (G2, G3, and G4), respectively. During the ten days of treatment, the number of oocysts decreased while the number of oocysts in the G2 increased steadily and reach the infection peak between 12 and 14 DPI, which recorded (9375-11000) OPG, at 12-14 DPI, G3 was (875-625) OPG while G4 reaches to (625-250)

OPG.

The number of oocysts decreased steadily in G3, G4, with a significant difference between the two groups on all experimental days; G3 reach (375-350) OPG on 16-17 DPI, respectively. The treatment of curcumin was able to cease oocyst shedding without a recurrence 10 days after medication withdrawal, whereas G4 achieved (125-0) OPG during the same periods and vanished after stopping therapy. G2 continuously shedding of oocysts for 16 days reach to 250 OPG at 23 DPI then stopped, while G3 reach to 125 OPG at 20 DPI, and G4 reach to 125 OPG at 16 DPI then stopped Table 1.

### HISTOPATHOLOGICAL EXAMINATION

G2 underwent histopathological analysis at 3 DPI, which revealed moderate intestinal villi shortening, sporadic *Cryptosporidium* oocysts presence at the apical surface with intact villus tissue without inflammatory cell infiltration (Figure 1).



**Figure 1:** G2 after 3 (DPI). *Cryptosporidium* oocysts (black arrow) and intact apical microvilli border demonstrated moderate shortening of intestinal villi.

### BEGINNING AT 8 DPI, WHEN OOCYSTS FIRST BEGIN TO SHED

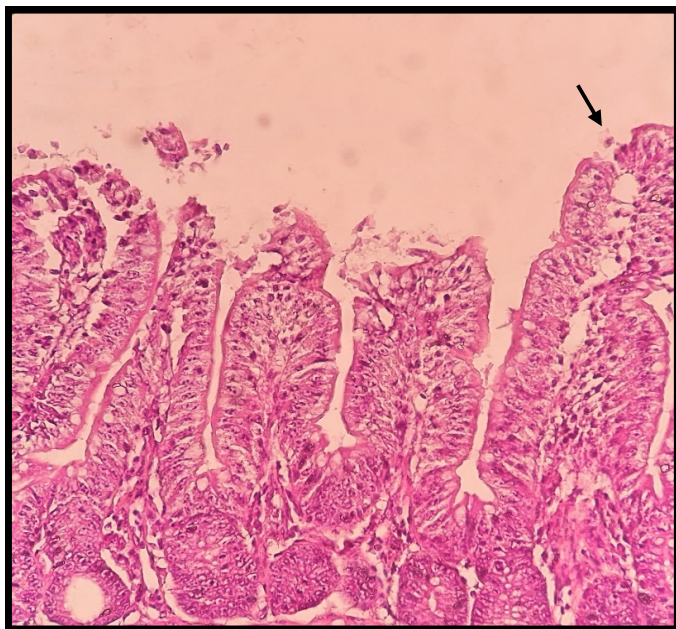
Histopathology assessment of G2 at 8 DPI showing massive atrophy, localized ulceration, epithelial sloughing, numerous oocysts adhered to the villi, and stunting of intestinal villi (Figure 2) with goblet cell hyperplasia were all visible in the intestinal ileum mucosa (Figure 3).



**Table 1:** the average number of *Cryptosporidium meleagridis* oocysts in each group during the 30 day experience.

dpi	dpt	G2 (Con+) Mean (Mean Rank)	G3 (paromomycin) Mean (Mean Rank)	G4 (curcumin) Mean (Mean Rank)	Chi- square (x <sup>2</sup> )	P-value
0-7	-	-	-	-	-	-
8	1	2000 (39.65±41)	2000 (125.50± 29)	2250 (85.50± 41)	147.02 **	0.0063
9	2	3700 (64.75±33)	1750 (115.50± 16)	1125 (73.35± 40)	172.38 **	0.0001
10	3	5500 (84.85±39)	1125 (104.70± 20)	1000 (67.65± 25)	185.62 **	0.0001
11	4	6500 (103.10± 33)	1000 (95.70± 21)	750 (54.75± 22)	207.75 **	0.0001
12	5	9375 (133.50)± 27	875(85.95 ± 13)	625 (46.25± 8)	186.56 **	0.0001
13	6	10250 (144.60± 42)	750 (75.35± 12)	375 (35.20± 15)	264.09 **	0.0001
14	7	11000 (155.40± 46)	625 (65.00± 17)	250 (21.65± 12)	178.41 **	0.0001
15	8	9000 (128.20± 27)	500 (55.40± 21)	250 (17.30± 7)	248.09 **	0.0001
16	9	7850 (115.80± 45)	375 (41.90± 15)	125 (7.85± 29)	187.55 **	0.0001
17	10	6375 (97.90± 37)	350 (38.80± 17)	-	179.02 **	0.0001
18		5250 (76.15± 34)	250 (25.80± 15)	-	186.71 **	0.0001
19		3500 (51.26± 34)	125 (10.95± 23)	-	155.37 **	0.0001
20		2100 (43.35± 36)	125 (10.95± 23)	-	147.07 **	0.0001
21		1125 (26.50± 30)	-	-	--	--
22		750 (16.50± 24)	-	-	--	--
23		250 (6.50± 32)	-	-	--	--
24-30	-	-	-	-	--	--
Chi-square (x <sup>2</sup> )		273.08**(157.40)	192.66**(126.76)	117.04**(85.90)	--	--
P-value		0.0001	0.0001	0.0001	---	---

\*\*P≤ 0.01

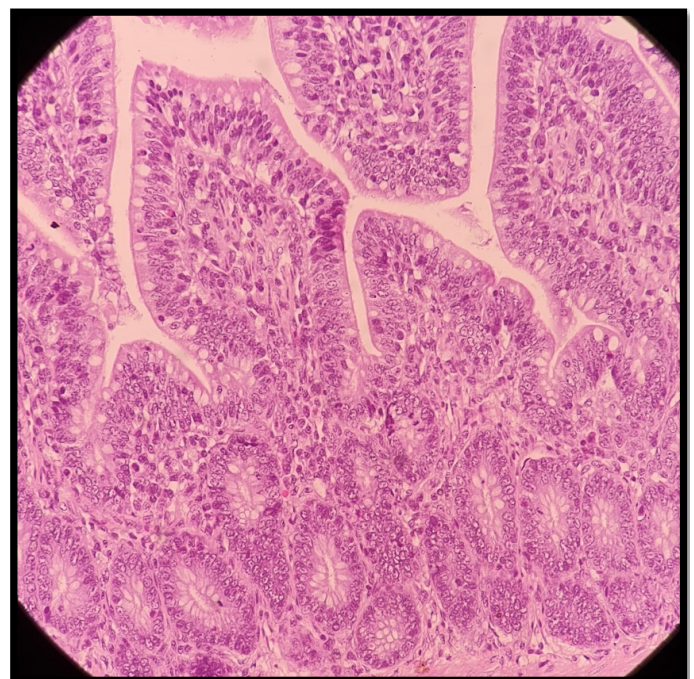


**Figure 2:** Ileum lesion on G2 (8 DPI), considerable villous shortening, epithelial sloughing acceptance, and multiple established stages of parasites were visible with several *Cryptosporidium* oocysts attached (black arrow), H & E stain X40.

#### AT THE PEAK OF SHEDDING (14 DPI)

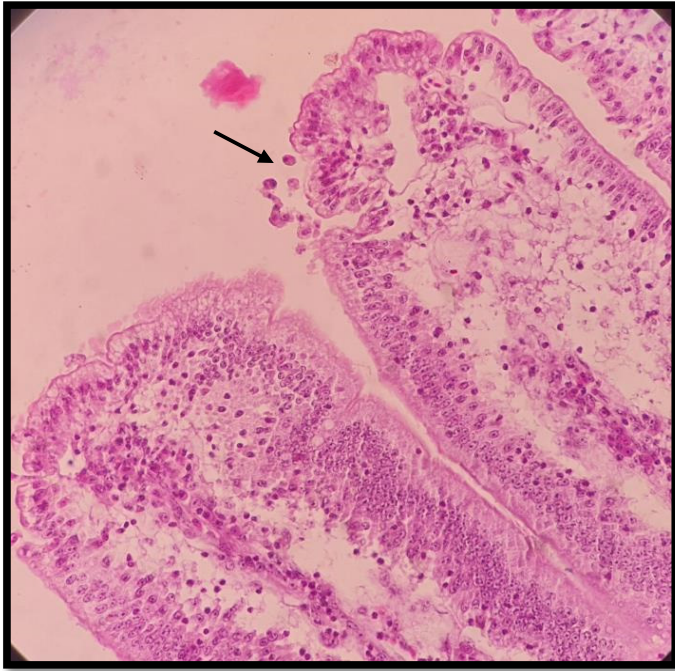
Histopathological evaluation of ileum on 14 DPI of (G2)

revealed the presence of a development stage at the brush boundary of intestinal villi enterocytes, which were visible as small, round or oval basophilic formations (Figure 4),

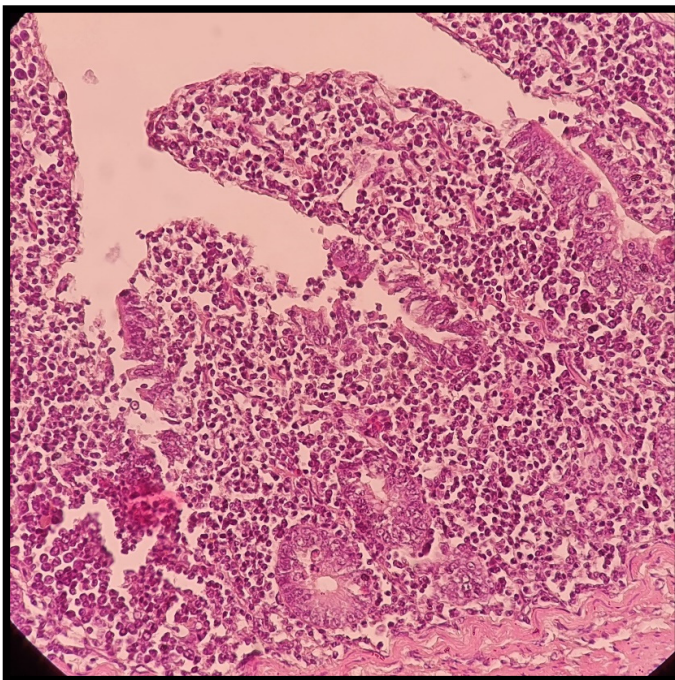


**Figure 3:** Ileum lesion on G2 (8 DPI); goblet cell hyperplasia with evident stunting of intestinal villi, (H & E stain X 40).





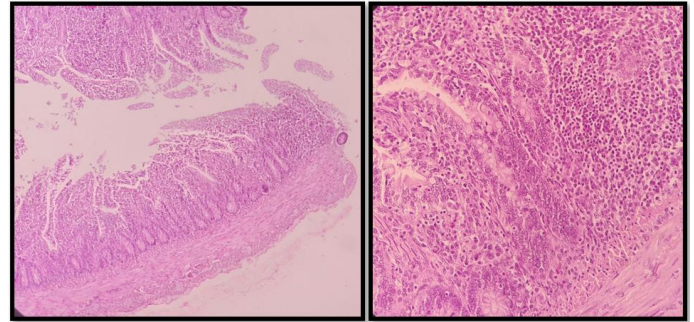
**Figure 4:** G2 (14 dpi), *Cryptosporidium* on the epithelial surface lining (oval round ball) (black arrow), with proprial distention tissue with leukocytic infiltration (H & E stain X 40).



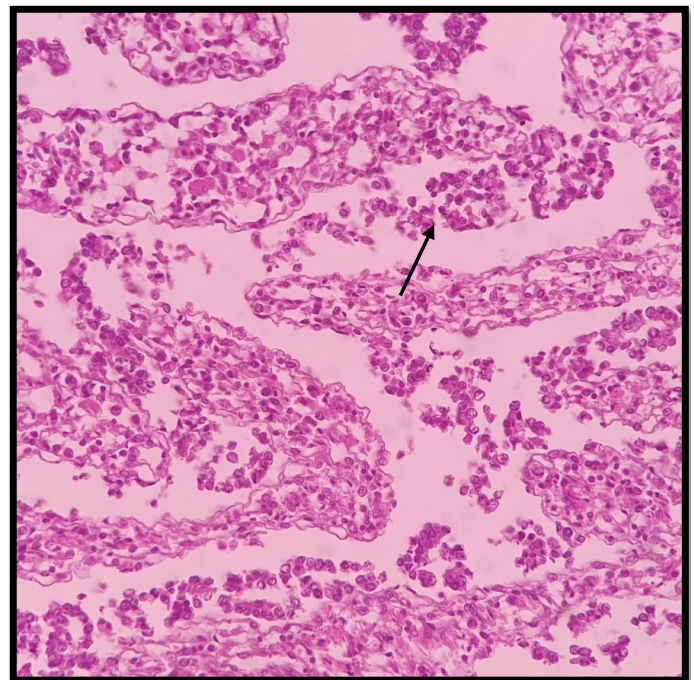
**Figure 5:** ileum examination on G3 (14 DPI), severe infiltration of many cell types with goblet cell hyperplasia, (H& E stain X 40).

G3 (14 DPI) revealed infiltration of several cell types (neutrophils and mononuclear cells) in the lamina propria together with goblet cell hyperplasia and multifocal necrotic findings, Crypt tissue manifestation revealed varying degrees of size reduction, considerable depletion of lymphoid- related tissue, apparent abnormalities of villus tissue linked with villous blunting and diffuse mononuclear

cell infiltration, and few to moderate oocysts are identified in the villi surface (Figure 5). G4 (14 dpi) indicated mild to minimal mononuclear infiltration in the mucosal and submucosal tissue, and the parasite was missing from the villi, the intestinal manifestation included indications of some villus and shortening, as well as an increase of the gut-associated lymphoid tissue, along with a partial improvement of the lesion (Figure 6).



**Figure 6:** Intestinal segment on G4 (14dpi), expansion of gut-associated lymphoid tissue, and an indication of some villus fusion and shortening, (H & E stain X 40).

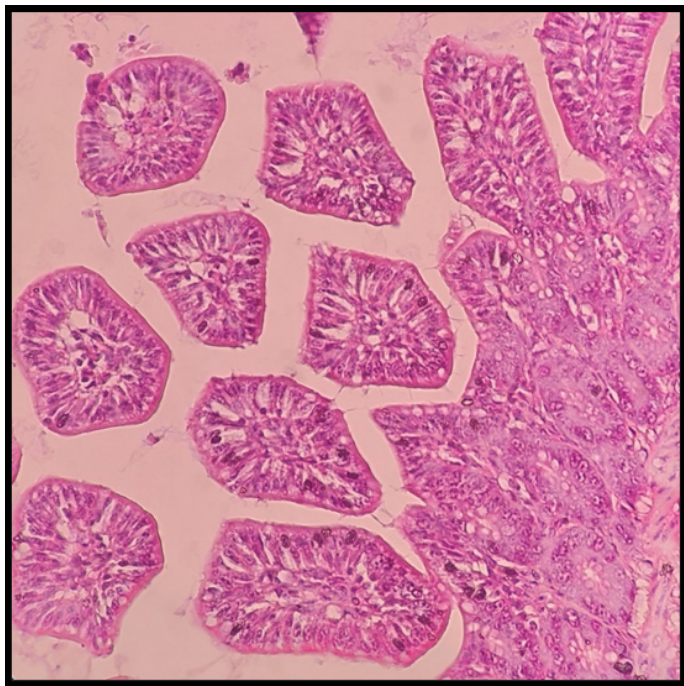


**Figure 7:** G2 (23 DPI) the mucosa and submucosal tissues were severely disrupted, and there were marked cystic crypt dilations and disorganized villus structures. A higher magnification of the previous section revealed the complete loss of enterocytes along with remnants of proprial tissue that still contained severe inflammatory cells, necrotic debris between villi (black arrow), (H & E stain X 40).

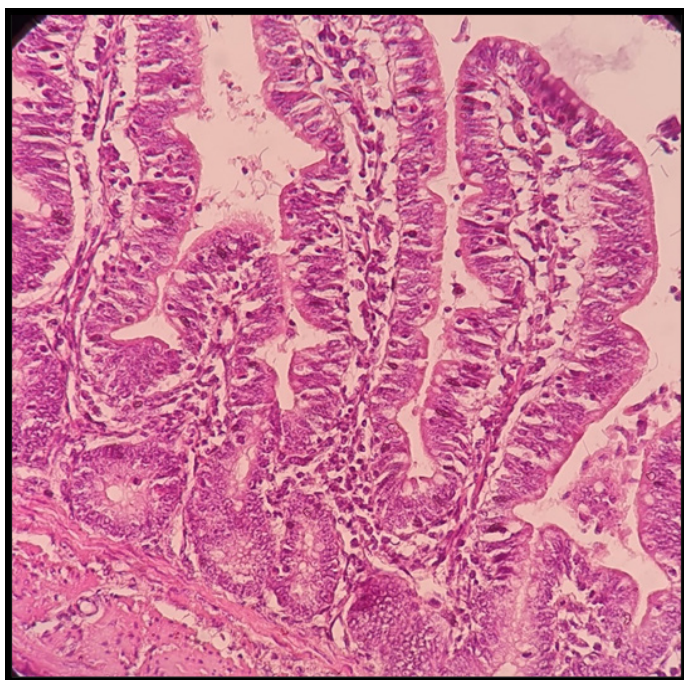
At the end of oocysts shedding (23DPI) G2 the intestinal tissue shows extensive mononuclear cell infiltration in the necrotic villi, along signs of various villus abnormalities, with a few *Cryptosporidium* are present as a rounded basophilic structure on the surface of the remaining villi



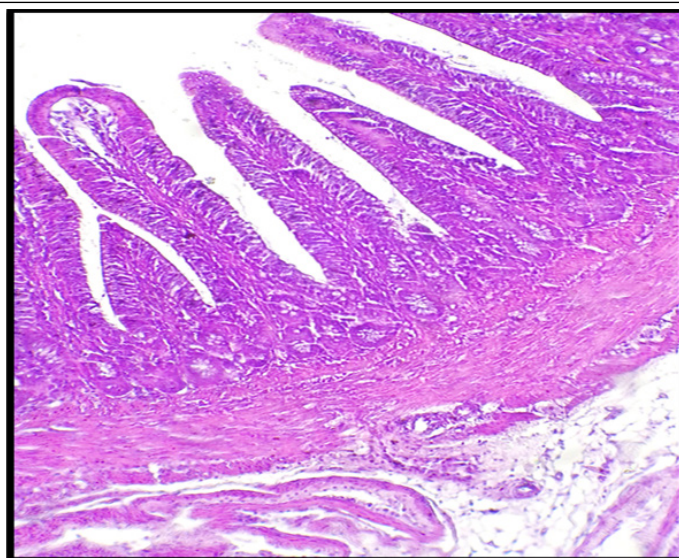
(Figure 7). At the same period on G3, intestinal tissue displayed mild to moderate goblet cell hyperplasia with no discernible changes in the mucosal and submucosal tissue surrounding it (Figure 8). In contrast, a section from a G4 bird showed mild mucosal mononuclear cell infiltration with normal crypt tissue (Figure 9). During all days of experiment, G1 and G5 showed no clear pathological alteration (Figures 10, 11).



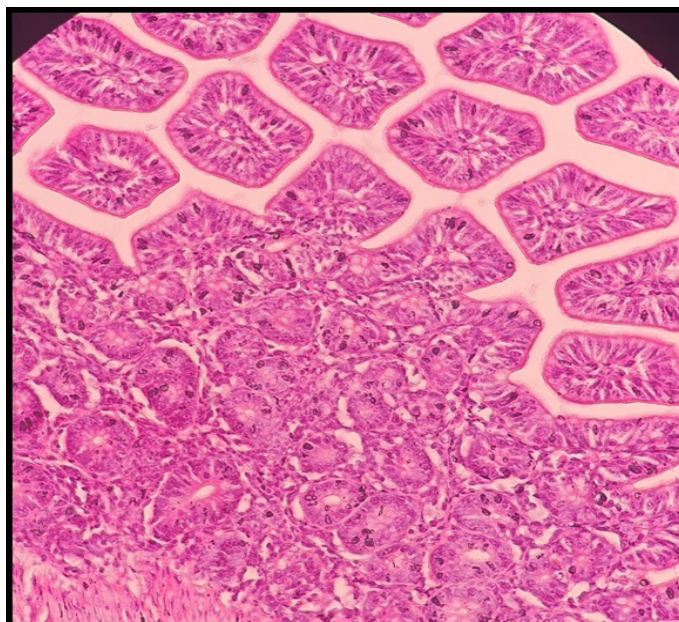
**Figure 8:** G3 ileum lesion (23 DPI) mild to moderate goblet cell hyperplasia without obvious alterations in the tissue around it, (H & E stain X 40).



**Figure 9:** Ileum section G4 (23 DPI) mild mucosal mononuclear cell infiltration with normal crypt tissue, (H & E stain X 40).



**Figure 10:** Intestinal section G1 normal intestinal structure, (H & E stain X 40).



**Figure 11:** intestinal section of G5 showed normal structural details of epithelial mucosa and lamina propria, (H & E stain X 40).

#### DRUG EFFECTIVENESS

The result showed no significant differences between curcumin and paromomycin, the effectiveness of curcumin was recorded 91.29% which higher than paraomomycin that recorded 87.29% Table 2.

**Table 2:** The drug effects of paromomycin and curcumin after ten days of treatment.

Drug type	Drug effectiveness
Curcumin	91.29%
Paromomycin	87.29%
Chi-square ( $\chi^2$ )	0.091NS
P-value	0.764

NS: Non- Significant.



During experimental infection of quails by  $1 \times 10^3$  oocysts per quail without mortality, This outcome is consistent with Al-Mahmood (2011) finding that  $1 \times 10^3$  oocysts are able to cause infection with notable oocysts shedding and histopathological alteration, with a 100% infection rate. That gives an indication about the ability of quails to infection in the early days of life that's due to an incomplete immune system and also due to the ability to infect with first time by high doses. And also agree with (Al-Khayat and Al-Zubaidi, 2015; Kadhim and Al-Zubaidi, 2018; Marwa et al., 2018; El-Shafei et al., 2018).

Because of the preferential side of *Cryptosporidium meleagridis* inside the intestine, experimentally infected birds typically remained asymptomatic or had moderate symptoms, this result agreement with (Al-Mahmood, 2011; Guechtouli et al., 2022). At 8 to 9 DPI, oocysts were initially noticed in quails droppings (P.P) this is due to the ability of quails for infection and the viability of oocysts to cause infection, and maximum oocysts shedding occurred at 14 DPI, this is in agreement with (Al-Zubadia et al., 2018; Cui et al., 2018; Kopacz et al., 2019; Holubov et al., 2020).

Oocysts shedding varies depending on the species of *Cryptosporidium* and the condition of the host; *C. meleagridis* is known for having low shedding, which is consistent with (Lin et al., 2022). On (G2, G3, and G4), the oocysts shed continuously for 16, 13, and 9 days, respectively.

Depending on the species of *Cryptosporidium*, the condition of test animals, and pathogenicity, different (p.p) exist for different *Cryptosporidium* species. The p.p in pigs is 10-12 days, in healthy humans 3-4 days, in old chickens 12-16 days, in young chickens 8-9 days, and in *C. avium* 8-9 days in hens and ducks. Oocysts excretion in feces begins roughly when symptoms begin and may last for a month. Species-specific differences exist in *Cryptosporidium* spp. preference sites. The development of *C. meleagridis*, *C. gill*, and *C. bailyia* is restricted to the villous epithelium of the terminal third of the small intestine, the proventriculus, and (trachea, bursa, and conjunctiva) respectively (Joker et al., 2021; Altamimi and Al-Zubaidi, 2021).

Before the experiment began, the weights of all quail ranged from (17-19g) at 7 days of age. During the infection, exactly after the fifth day of infection, weight gain in the infected group G2 was significantly reduced from (17-19g) to (14-16g), whereas the weight of both treatment groups was increased to (25-30g) and (70-75g) in G3, G4, respectively. It differs considerably from the control groups G1 and G5, which weigh, respectively (95-100) and (98-120g). that's due to reduced observation surface which leads to histological alteration that disagrees

with Guechtouli et al. (2022) how found that the clinical signs in birds infected with *C. baliayi* were non-specific signs, normal rales were 28%, cachexia was 22% and diarrhea was 20%.

### ANTICRYPTOSIDIAL EFFECT OF PAROMOMYCIN AND CURCUMIN

When treatment began on the eighth day of infection, the number of oocysts in one gram of drops was (2000, 2000, and 2250) oocysts per gram (OPG) in (G2, G3, and G4), respectively.

The number of oocysts decreased steadily in G3, G4, with a significant difference between the two groups on all experimental days; G3 reach (375-350) OPG on 16-17 DPI, respectively. The treatment of curcumin was able to cease oocyst shedding without a recurrence 10 days after medication withdrawal, whereas G4 achieved (125-0) OPG during the same periods and vanished after stopping therapy. Paromomycin did not completely eradicate the cryptosporidial infection in quails, and there was a strong link between treatment days and a decrease in oocyst shedding in both treatment groups, this result was in agreement with Asadpour et al. (2018) who noted that curcumin anticryptosidial effect was more rapid than paromomycin and required further investigation, different pharmacological qualities including antioxidant, antiprotozoal, and anti-inflammatory activity in conjunction with curcumin, which has aid in the treatment of Cryptosporidiosis and its clinical lesion. In the experiment, oocyst shedding, histopathological alteration, and medication efficacy were used to assess the anti-cryptosporidial potential of curcumin in quails. This finding is in agreement with the earlier studies that demonstrated that paromomycin cannot completely eradicate cryptosporidial infection (Van Zeeland et al., 2008; Obaid et al., 2012). Curcumin's medicinal properties include its polyphenolic curcuminoid compounds (diferuloylmethane), which have the advantage of remaining inside the gut for an extended period of time. These compounds also have an impact on oocyst viability and sporozoite morphology by affecting gene transcription, and they inhibit intracellular adhesion molecules, which are involved in the sequestration and establishment of aporozoites and agreement with Kadir et al. (2008) how found that the medical plant extracts have an effect on cryptosporidiosis in experimental infected mice.

### DRUG EFFECTIVENESS

The result showed no significant differences between curcumin and paromomycin, there are numerous variables that affect a drug's effectiveness, including an individual's age, sex, body weight, and general health. The medicine had a considerable impact on clinical symptoms (changed

feces to the usual form, decreased the number of days that oocysts shed, improved food intake, raised body weight, and the tissue improvement was evident in the histology analysis).

### HISTOPATHOLOGICAL EXAMINATION

Histological examination changes in this study were agreed with (Al-Mahmood, 2011; Marhoon and Jasim, 2017; Al-Zubaidi et al., 2018; Kadhim and Al-Zubaidi, 2018; Asadpour et al., 2018). The Same result was obtained from Al-Zarkoushi and Al-Zubaidi (2021b) on quails intestinal tissue that was infected with coccidian. Al-Warid et al. (2014) concluded that lymphocyte and phagocytosis are good indicator to evaluate the cellular immune response in experimentally mice with *Cryptosporidium* spp. This result disagreed with Cui et al. (2018) who found numbers of *C. avium* oocysts cover the epithelial cell of the small intestine without any pathological change in the epithelial cell.

When treated to infected quail, paromomycin and curcumin both displayed anticryptosidial activity; however, curcumin was more successful in curing lesions and lowering the number of oocysts on the villi, but paromomycin was unable to stop infection, oocysts shedding, or improve affected tissue (Aydogdu et al., 2018). Contrary to paromomycin, among other things, inhibits protein synthesis by binding to *16 sr RNA*, curcumin's therapeutic effect are also related to changes in the gut microbiota and genes associated with the innate immune system, plant extract have an effective result in the treatment of infection (Hazaa et al., 2016). Paromomycin only affects the parasite and has no effect on the colonization or improvement of tissue (Fuloria et al., 2022).

### CONCLUSIONS AND RECOMMENDATIONS

Our research revealed that curcumin was a reliable option against cryptosporidial infection, as it significantly reduced the infection load in experimentally infected quails and completely eliminated the infection without recurrence after medication removal.

- Further studies are needed to clarify the mechanism by which curcumin induces apoptosis in *Cryptosporidium*-infected cells.
- Further studies are necessary to examine the therapeutic potential of curcumin in other animal models and also in immunosuppressed individuals.
- More studies on *Cryptosporidium* spp. subtypes and determination of zoonotic types between humans and chickens and determinants of the epidemiology factor with the mode of transmission.

### ACKNOWLEDGMENTS

I would like to thank prof. Dr. Mohammed Thabit for his flexibility, professional ideas, and advice throughout my study and to the staff of the Parasitology Department/ College of Vet. Med. Baghdad University.

### NOVELTY STATEMENT

Cryptosporidiosis is a fatal disease in quail and there is no effective drug on it, therefore looking for safety substance, anti-proliferative, anti-microbial, anti-inflammatory, anti-oxidant properties, and anti-protozoal. Numerous drugs and ingredients have been investigated as potential treatments for the condition, but there is presently no pharmaceutical that can cure Cryptosporidiosis, thus further work is required to create a potent therapy. There is no data available about quails cryptosporidiosis in Iraq.

### AUTHOR'S CONTRIBUTION

Conceived and planned the experiments contributed to sample preparation. Contributed to the interpretation of the results.

### ETHICAL STATEMENT

All animals handled in the current experimental study were used according to the ethics of the local committee of animal care and use at the College of Veterinary Medicine within the University of Baghdad (Number 2569/PG on 12/12/2022).

### CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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