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RESEARCH ARTICLE

Prevalence, Morphometric, Genomic and Histopathological Studies in Backyard Chickens Coccidiosis in Soran City, Erbil-Iraq

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ABSTRACT

Coccidiosis is an intestinal parasitic disease of chickens caused by the intracellular parasite *Eimeria* spp. It is responsible for high rates of mortality and morbidity in the poultry industry worldwide. It has a significant economic impact on poultry production. A cross-sectional study conducted in Soran City/Erbil, Iraq from June 1, 2021, to May 31, 2022, aimed to identify the prevalence, seasonality, and species characterization, of *Eimeria* spp. using 18S rDNA, and histopathological changes associated with *Eimeria* infection in Backyard chickens. In total, 400 intestinal scraping and fecal samples were examined, out of which 165 (41.25%) were found to be infected with four species of *Eimeria*. The highest prevalence of *Eimeria* was observed in winter at 30.3%, followed by autumn at 29% and spring at 27.2%. The summer season had the lowest number of cases only 13.3%. A morphometric analysis was carried out on sporulated oocysts to determine the species of *Eimeria*. The analysis detected four species of *Eimeria*, with decreasing prevalence as follows: *Eimeria tenella* (25%), *E. necatrix* (20%), *E. acervulina* (15%), and *E. brunetti* (7%). Additionally, mixed infections were found in 33 cases mainly *E. tenella* + *E. necatrix*. The amplified gDNA from oocysts using genus-specific primers targeting 18S ribosomal RNA revealed 455 bp gave the genus confirmation. The histopathological study clarified that the invasion of the parasite to the intestinal layer causes damage and necrosis as well as provokes immune responses that were presented by different inflammatory cells and infiltration mainly lymphocytes, plasma cells, and eosinophils.

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INTRODUCTION

Poultry is an essential source of animal protein, playing a significant role in meat and egg production. The demand for this type of protein is increasing rapidly worldwide.

The Eimeriorinid coccidia is a group of apicomplexan protozoan parasites that comprises various pathogens of veterinary and medical significance. These pathogens include species belonging to the Eimeriidae and Sarcocystidae families, for instance, *Eimeria* spp. and *Sarcocystis* spp, respectively (Ogedengbe *et al*., 2011; Swar and Shnawa, 2020).

The protozoan disease known as coccidiosis is a type of intracellular parasite that has a major impact on the production of poultry and is considered economically significant (Jebessa *et al*., 2022). The seven *Eimeria* species live in different parts of the gastrointestinal tract and can cause poultry diseases, ranging from mild enteritis to severe mortality. The severity of coccidiosis varies depending on factors such as the *Eimeria* species, strain, infectious dose, the genetic makeup of the host, flock density, environmental and stress conditions, and other infections that may be present (Bachaya *et al*., 2015; Ahmad *et al*., 2024).

Coccidiosis is a severe enteric disease that affects the poultry industry globally. It is caused by species belonging to the genus *Eimeria*. Seven known species of *Eimeria* affect chickens, namely *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*. These species demonstrate a robust site-specificity of development within the intestines of birds (Dubey *et al*., 2020; Jebessa *et al*., 2022). *E. mitis* and *E. necatrix* have a preference for ileum and jejunum, while *E. tenella* parasitizes the caecum. The pathogenicity of different species varies, and *E. tenella* is known to be the

most virulent species responsible for significant mortality. *E. necatrix* is highly virulent, causing a chronic form of chicken coccidiosis. (Kundu *et al*., 2020). Results from recent studies have indicated that when they infect the same host, *E. mitis* and *E. tenella* /*E. necatrix* can have a synergistic relationship. The presence of *E. mitis* can intensify the disease pathology caused by *E. tenella* and may also increase the impact of *E. necatrix* in co-infections (Xu *et al*., 2022; Abbas and Alkheraije, 2023).

The widespread nature of these parasites and their growing resistance to drugs make the situation even more serious (Abbas *et al*., 2011a; Al Syaad *et al*., 2023). Nowadays, alternative approaches including nanoparticles and botanical materials have been established as an important breakthrough in the treatment and management of parasitic diseases including protozoal infections (Abbas *et al*., 2010, 2011b, 2011c, 2012, 2013, 2017, 2019; Zaman *et al*., 2012; Idris *et al*., 2017, Mohsin *et al*., 2021). The field of parasite control through the use of nanomedicine has made significant progress in recent years (Abbas *et al*., 2020, 2022; Shnawa *et al*., 2021, 2023). But, despite all these integrated approaches to control coccidiosis, this disease is highly prevalent in most parts of the world.

The purpose of this study was to investigate the prevalence and seasonality of *Eimeria* in backyard chicks in Soran City, Erbil-Iraq. As well as the histopathological effects of this parasite were also elucidated, due to the scarcity of information on chicken *Eimeria* species in this area.

MATERIALS AND METHODS

Collection of coccidian parasite: A total of 400 backyard chicken (*Gallus gallus*) guts samples were freshly collected from slaughtered poultry shops in Soran, Erbil, Kurdistan-Iraq. All samples were collected randomly for one year, during the period from June 1, 2021, to May 31, 2022, each month about 35 chicken samples. The samples were then transferred to the laboratory of the biology department, faculty of sciences of Soran University. The intestinal contents were microscopically examined to estimate the percentage of the naturally infected chickens with coccidiosis. The oocysts of *Eimeria* spp. were collected depending on the technique described by Abbas *et al* (2015). Ten grams of chicken feces or scraped samples of intestinal materials were dissolved in 10 ml of tap water. The resulting solution was then filtered using two layers of gauze and precisely transferred to one or more centrifuge tubes, depending on the volume. The tubes were then centrifuged at 3000 rpm for 5 minutes. After centrifugation, the supernatant was carefully removed, and all the sediments were transferred to one tube. This tube was then subjected to a second round of centrifugation at 3000 rpm for 5 minutes, then slides were prepared. Photomicrographs of the oocysts from each sample were captured and analyzed for their morphometric characteristics using a Leica microscope that was linked to a camera (Leica DM2700, Germany). The mean (range) values were calculated and presented as output.

Sporulation: Samples that appeared positive for oocysts were diluted in a 2.5% aqueous solution of potassium dichromate (K2Cr2O7) and placed in Petri dishes to allow for sporulation. The Petri dishes were partly covered to allow the passage of oxygen and incubated at 25-29º C for 48 hrs, providing 60-80% humidity and maintained by keeping water in two Petri dishes in the incubator. Following sporulation, the oocysts were retrieved through centrifugation using a saturated sugar solution, following the method designated by Duszynski and Wilber (1997). The sporulation of the oocysts was detected by investigating sporocysts under the microscope at 40x (Abbas *et al*., 2015).

Additionally, the oocyst shape index values were calculated and compared with the standard diagnostic guide provided by Conway and McKenzie (2007). This comparison helped to identify the species encountered in the study. Identification of different species was achieved based on various characteristics such as oocyst morphology, sporulation time, the zone of the intestine parasitized and parasite location in the host intestinal epithelium.

The sporulated oocysts were distinguished by their size and shape through the measurement of 25 oocysts at 400x magnification with a calibrated ocular micrometre. These oocysts were classified into three groups, namely the *Acervulina-Mitis* (AM) group, Necatrix, *Tenella & Praecox* (NTP) group, and *Brunetti-Maxima* (BM) group, based on the classification system of Haug *et al*. (2008). The Acervulina-Mitis group consisted of small oocysts (<18.8µm) that were tentatively identified as *Eimeria acervulina* and/or *Eimeria mitis*. The NTP group consisted of medium-sized oocysts (18.9 to 23.8 µm) that were tentatively identified as *Eimeria necatrix*, *Eimeria tenella* and/or *Eimeria praecox*. Finally, the BM group consisted of large ovoid oocysts (>23.9µm) that were tentatively identified as *Eimeria brunetti* and/or *Eimeria maxima* (Haug *et al*., 2008).

Histological section preparation: Various portions of the small intestine, comprising the duodenum, jejunum, and ileum, in addition to the large intestine (caeca), were gathered for thorough assessments utilizing both macroscopic and histopathological analyses. Visible anomalies were inspected in all sections. All the segments were fixed with 10% neutral buffered formalin for 24 hours. After this, paraffin-embedded blocks were prepared. The processed sections were sectioned into five-micron sections using a microtome, and stained with hematoxylin and eosin (H&E). The stained sections were scrutinized and evaluated using a light microscope (Slaoui and Fiette, 2011).

Genomic DNA extraction: Genomic DNA was extracted from oocysts of *Eimeria* spp. using the FavorPrep™ Stool DNA Isolation Mini Kit (Favorgen, Biotech Corp. Taiwan) following the manufacturer's protocol, as follows: A mixture of 200 mg of Glass Beads and 100mg of stool sample was prepared in a 2.0ml Bead Tube. SDE1 Buffer and proteinase K (10 mg/ml) were added to the mixture and vortexed for 5 minutes. The sample was then incubated at 70°C for 10 minutes. SDE2 Buffer was added to the sample followed by centrifugation at 14,000 rpm for 5 minutes. The resulting supernatant was transferred to a microcentrifuge tube. After several steps, the SDE Column was placed into an Elution Tube to elute DNA.

The PCR assay of *Eimeria* spp. 18S rDNA: The DNA was additionally verified by procedure targeting of the 18S rRNA gene using *Eimeria* genus-specific primers (Forward primer: 5′-CGCGCAAATTACCCAATGAA-3′ and reverse primer: 5′-ATGCCCCCAACTGTCCCTAT-3′) (Hinsu *et al*., 2018) resulting in an amplicon of ~ 455-465 base pairs. The gDNA was used as a template for PCR amplification. Each 20 µL PCR reaction mixture comprised of 3 µL gDNA, 1 µL of each forward and reverse primer, 10 µL Master Mix and 5 µL DNase, RNase-free water.

The PCR (GeneAmp*, PCR System 9700, Applied Biosystem, USA) was used, the PCR amplification cycles were: initial denaturation at 95ºC for 5 min, followed by 35 cycles of 95ºC for 30 s, 60ºC for 45 s and 72ºC for 45 s, and a final extension at 72ºC for 10 minutes. The PCR product was analyzed with Gel electrophoresis to observe the bands of the DNA related to the (18S rDNA) in 20ml of 5X TBE buffer per 80 ml of distilled water and 1.5% (w/v) of agarose with 8 µL SYBR Safe dye and it was performed at 90 voltage for 60 minutes.

Statistical analysis: SPSS 26 was used to generate graphs and statistics. To identify research features and outcomes, a one-sample t-test was employed to report variables as frequencies and percentages. In addition, the Kruskal-Wallis and Friedman tests were used to compare the prevalence of different groups. When the p-value was less than or equal to 0.05, statistical significance was assumed.

RESULTS

In the present work, 400 backyard chickens were examined for coccidiosis infection, 165 (41.25) chickens were infected with the *Eimeria* species, and 235 (58.75) of them appeared to be negative (Fig. 1 and 2).

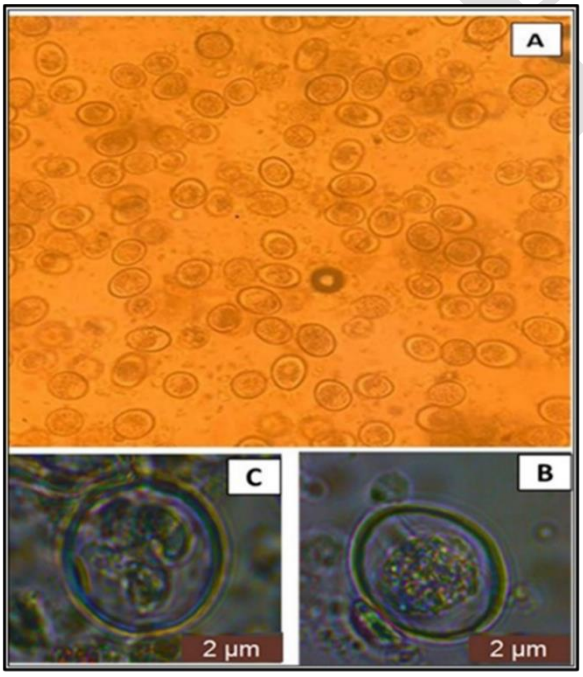

Fig. 1: A. Microscopic examination of intestinal scraping of infected chicken showing **o**ocysts of *Eimeria* spp X400 B. Unsporulated oocyst of *Eimeria* spp. Scale bar = 2µm.C. Sporulated oocyst of *Eimeria* spp. showing an oocyst wall with outer and inner layers containing four sporocysts. Scale bar=2µm.

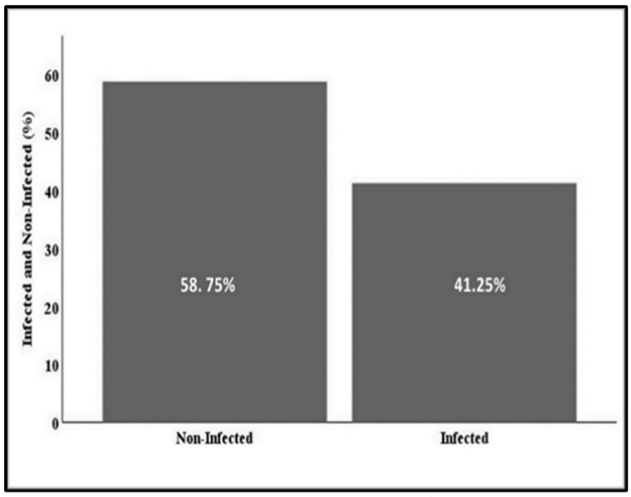

Fig. 2: Prevalence of *Eimeria* spp. infection in chickens (P<0.001).

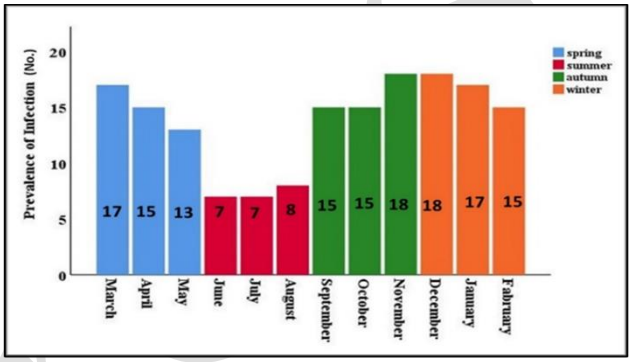

Fig. 3: Prevalence of *Eimeria* infection by months and by seasons (X2= 18.67, P< 0.001).

According to the findings regarding seasonality, out of the 165 chickens that were infected, the majority of the infections took place during the winter with 50 cases (30.3%), followed by autumn with 48 cases (29%), and spring with 45 cases (27.2%). In contrast, the least number of cases were recorded in the summer, with only 22 cases (13.3%), as illustrated in Fig. 3. Moreover, our study found that there were more infections during November and December, with 18 cases reported for both months. This was followed by January and March, which had 17 cases each. The remaining months recorded lower numbers of infections, which were arranged in descending, as illustrated in Fig. 3.

Four distinct types of *Eimeria* spp. were detected during the analysis of the morphometric characteristics as illustrated in Table 1 and Fig. 4, with *E. tenella* being the most prevalent (25%), followed by *E. necatrix* (20%), *E. acervulina* (15%), and *E. brunetti* (7%). Furthermore, mixed infections were found in 54 cases, mainly *E. tenella* and *E. necatrix* in 31 of the infected chickens.

Moreover, all of the samples that were considered positive through morphological examination yielded electrophoretic bands. Bands detected at the 455 bp region in gel electrophoresis of PCR product of the 18S rRNA gene belonged to *Eimeria* spp. As depicted in Fig. 5.

The histopathological study: The histopathological examination of the intestinal parts of the infected chickens exhibited (41.25%) *Eimeria* infection. The tissue sections

Table 1: Prevalence and morphometric description of *Eimeria* spp.

Eimeria species	Number (%) of infection	Morphology of oocyst	Oocyst size (µm)	Shape index	Sporulation Time (hr.)	Location of infection
E. acervulina	25 (15)	Ovoid with double-layered walls	18.70×14.85	1.25	18	Small intestine (duodenum)
E. tenella	41 (25)	Ellipsoid ovoid double-layered walls	22.20×19.20	1.15	21	Caecum
E. brunetti	12 (7)	Ovoid double-layered walls	24.10×19.30	1.24	20	Small intestine (ileum)
E. necatrix	33 (20)	Oblong, ovoid double-layered walls	19.85×17.05	1.16	19	Small intestine (jejunum) and caeca.
E. tenella & E. *necatrix*	31 (19)	-	-			Small intestine and caecum
E. necatrix& *E. brunetti*	12 (7)					
E. acervuline & *E. brunetti*	11 (7)					
Total	165 (100)					

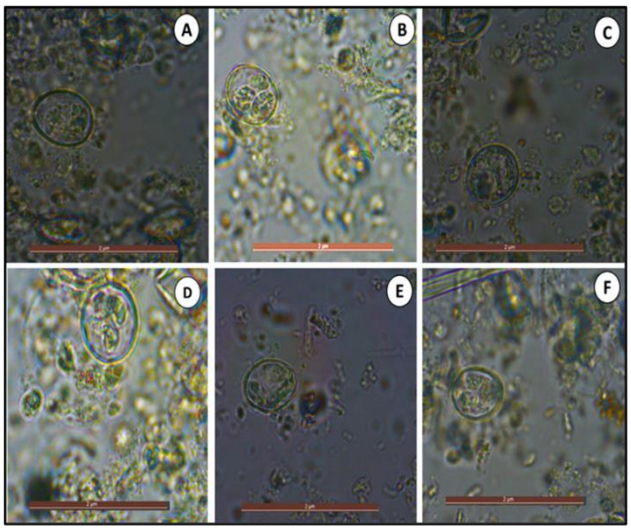

Fig. 4: Sporulated oocysts of *Eimeria* spp. A and B: *E. tenella,* C: *E. necatrix,* D: *E. brunetti,* E and F: *E. acervuline.*

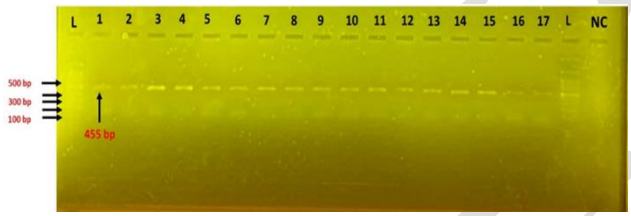

Fig. 5: The amplification product size of ≈ 455 bp of the 18S rRNA gene belonging to the genus *Eimeria* was displayed via agarose gel electrophoresis. The positive samples were loaded into wells 1-17, whereas the negative control was loaded into well NC. Marker 100 bp was used as a reference of molecular weight (100 bp DNA ladder) in the well L.

had parasitic structures compatible with *Eimeria* spp. in the duodenum, the jejunum, the cecum, and the colon. Different stages of the *Eimeria* parasite were observed in the intestinal mucosa and submucosa. The developmental stages of the parasite appeared in schizonts, macrogametes, microgametes, and immature oocysts. Some cases showed severe inflammation with different inflammatory cells, also eosinophils were detected remarkably in most examined sections. Regarding the infection of the cecum, our results showed the presence of the parasite within the glands of the crypts, as illustrated in Fig. 6 (A, B, C and G). Also, Fig. 7, B illustrates lymphoid-follicular aggregates of chronic inflammatory cells (lymphocytes) which is evidence of chronic inflammation. In addition, the damaged villus of

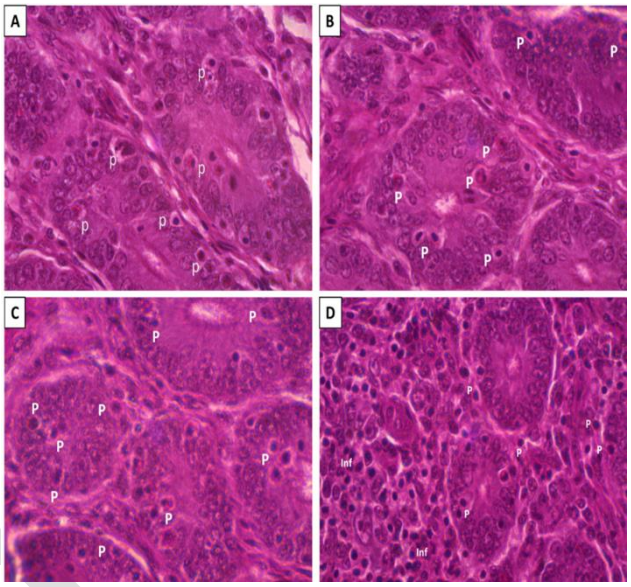

Fig. 6: A, B, C & D, Photomicrograph of the cecum of infected chicken show intraepithelial lymphocytes in the glands, parasites (P) and the presence of inflammatory cells infiltration (Inf). C. Section of the cecum of infected chick showing hyperplasia of the intestinal crypt lining epithelium associated with the presence of coccidian schizont stage (P). The crypts lining the epithelium are heavily infected with various stages of coccidian parasites. D. Heavy infiltration of inflammatory cells (Inf) with the presence of the parasite (P). D. Intestinal crypts, showing heavy infection of lining epithelium with different forms of coccidian parasite. H &E staining bar = 20 µ.

the duodenum explains the presence of parasites surrounded by necrosis and inflammatory cells mainly as lymphocytes during its passage via the lamina propria to the crypt epithelium. A parasite and mixed inflammatory cells and eosinophils, intra-epithelial leucocytes were also illustrated in Fig. 8 A, and the occurrence of the parasite with eosinophils and heterophils was clear in B. Furthermore, the sporozoite of *Eimeria* was in C with necrosis and inflammation, while giant cells were detected that formed by the fusion of several cells with multiple nuclei. Moreover, Fig. 9 confirms the presence of the parasite in the serosa and muscular properia, infiltration deep through the wall of the intestine, and lymphocytes with severe inflammation (lymphocytes and plasma cells), necrosis of a cellular area and occurrence of immature oocysts of *Eimeria* parasite and shows the parasites within the gland of the cecum with dense infiltration of inflammatory cells mainly lymphocytes.

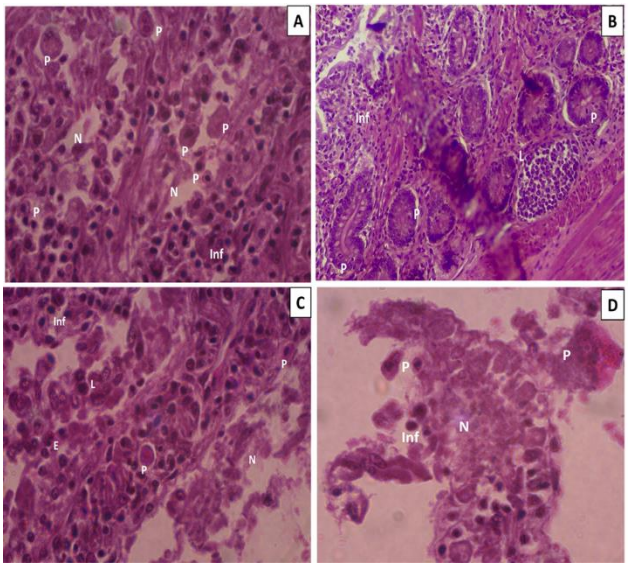

Fig. 7: A. Small intestine sections of infected chickens demonstrate the presence of the parasite (P) in all images. B. Illustrates Lymphoid-follicular aggregates of chronic inflammatory cells (lymphocytes) (L) mark of chronic inflammation. C. Shows necrosis (N) of epithelial tissues along with inflammatory cells and parasite stages (P) bar = 20µ. D. damaged villus of duodenum shows the presence of parasites (P) surrounded by necrosis (N) and inflammatory cells (Inf) mainly as lymphocytes. During its passage via the lamina propria to the crypt epithelium. H& E staining, bar = 10µ.

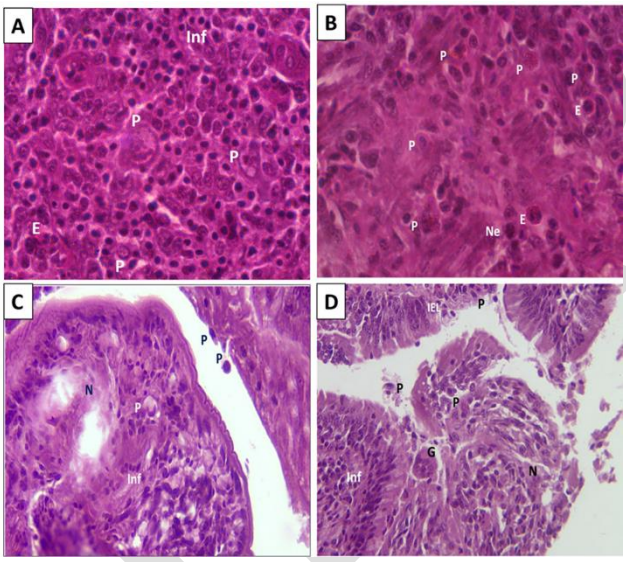

Fig. 8: Different stages of *Eimeria* parasite (P) and mixed inflammatory cells and eosinophils (E), intra-epithelial leucocytes. B. Explain the occurrence of the parasite with eosinophils (E) and Heterophils (neutrophils) (Ne). C. Showing the sporozoite of *Eimeria* (P) with necrosis and inflammation. D. Formed by the fusion of several cells, giant cells showing multiple nuclei when viewed under a microscope (G), (IEL) intra-epithelial leucocytes along with inflammation (Inf) and necrosis (N). H & E, bar = 20µ.

DISCUSSION

The poultry industry is still facing a major challenge due to coccidiosis, which is caused by various species of *Eimeria* parasites (Al Syaad *et al*., 2023).

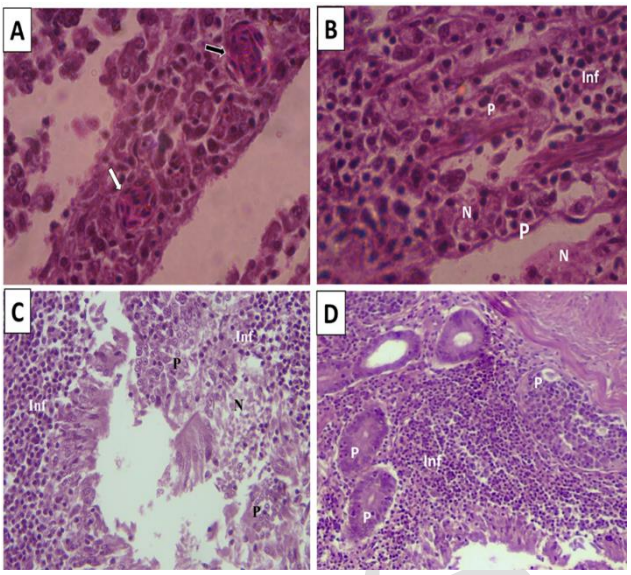

Fig. 9: A. Damaged mucosa exhibited section in mature schizont with merozoites (white arrow), raptured schizont with 2nd generation of merozoites (black arrow) and infiltration with inflammatory cells, H &E, bar = 10 µ. B. Serosa and muscular properia, infiltration deep through the wall of the intestine, and lymphocytes C, Severe inflammation (lymphocytes and plasma cells), necrosis of a cellular area and occurrence of immature oocysts of *Eimeria* parasite (P). D. Shows the parasites (P) within the gland of the colon with dense infiltration (Inf) of inflammatory cells mainly lymphocytes. H& E, bar = 20µ.

Out of the 400 chickens tested, our study showed that 165 of them were found to be infected with the various *Eimeria* species. Among these, 111 chickens were infected with a single species, while 54 chickens were infected with two or more species, with *E. tenella* and *E. necatrix* being the most common species. So our study demonstrated mixed infection with *Eimeria* spp., recently, A conclusion was drawn that experimental co-infection models demonstrated a synergistic relationship between *E. mitis* and *E. tenella* /*E. necatrix*. This proposes that the common natural mixed infection of chicken coccidia in the field was likely due to the synergistic effect of *Eimeria* spp. rather than an antagonistic one (Xu *et al.*, 2022). This might shed light on the fact that the presence of one *Eimeria* species exacerbated the consequence of co-infection with another *Eimeria* species and led to high mortality, intestinal lesion impact, and oocyst formation. Therefore, precise recognition of *Eimeria* spp. is crucial not just for identifying diseases but also for dealing with subclinical infections, creating and implementing effective control methods, and conducting biological and epidemiological research (Kumar, 2014).

In our study, *E. tenella* was found to be the most prevalent species among the recorded infections. It constituted 25% as a single infection, as well as 19% as mixed infections. This agrees with the survey conducted in Saudi Arabia to determine the incidence of *Eimeria* infection in backyard chicks raised in farms and houses. *E. tenella* was detected in house-reared chicks and had an 80% infection rate (Al-Quraishy *et al.*, 2009). In addition, Mares *et al*. (2023) recorded infection with *Eimeria* spp, *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, and *E. praecox*, as 10.84, 5.84, 4.16, 2.5 and 1.66% respectively. Also in Egypt, the highest prevalence rate was observed in

E. acovullina, followed by *E.tenella*, *E.necatrix*, and finally *E.mitis*. Their findings suggest that coccidiosis is a severe parasitic disease that significantly impacts poultry production (Mohamed *et al*., 2021).

Concerning the seasonality, our findings indicated that the majority of the infections occurred during the winter, followed by autumn, and spring. In contrast, the least number of cases were recorded in the summer. Similarly, Mohamed *et al*. (2021) found that chickens in Egypt were more susceptible to coccidiosis in winter compared to summer. This suggests that the incidence of coccidiosis in chickens is affected by seasonal changes. Also, coccidiosis was observed to be highest in autumn in Kashmir Valley, India (Ahad *et al*., 2015). Although oocyst sporulation and survival are more likely to occur in environments with higher humidity levels, particularly after the primary rainy periods, it is not feasible to attribute high or low *Eimeria* disease occurrence solely to climatic factors, even raised temperature proposed inhibitory environment for sporulation. Insufficient awareness of transmission and control, as well as limited resources, are also significant factors (Attree *et al*., 2021).

In contrast, another study related to broilers raised in the Nineveh governorate of Iraq revealed seasonal effects, with the highest incidence of caecal coccidiosis occurring in spring, followed by autumn, winter and summer seasons (AL-Taee, 2007).

Analyzing the oocysts' morphology and macroscopic lesions was used to identify the *Eimeria* species. Also, genus-specific PCR (18S rDNA gene) was performed on all DNA samples that tested positive as previously achieved (Soares Júnior *et al*., 2023). The diagnosis of coccidiosis was effectively established by histopathological examination, unrelatedly to the level of parasitism. In the diagnostic routine, histological lesion scores can be utilized. The most appropriate method for diagnosis is the combination of histopathological analysis and macroscopic evaluation. Also, the inclusion of PCR is necessary to assist in the identification of the seven *Eimeria* species present in broiler chickens (Balestrin *et al*., 2022).

Regarding the infection of the cecum and colon, our results showed the presence of the parasite within the glands of the crypts, as illustrated in Fig. 7 (A, B, C and D). This agrees with a previous study which mentioned that the *E. tenella* sporozoites do not directly enter the enterocytes of the crypts. Instead, they are carried to their development site by host cells. The host cells responsible for this transportation are intraepithelial lymphocytes, not macrophages, as previously thought. The evidence presented supports the idea that sporozoites initially penetrate surface enterocytes before entering intraepithelial lymphocytes, which then exit the epithelium, pass through the lamina propria, and enter the crypts (Lawn and Rose, 1982; Del Cacho *et al*., 2014).

This agrees with Al-Zarkoushi and Al-Zubaidi (2021) who previously determined that Quail coccidiosis exhibits histopathologic lesions which are characterized by severe necrotic enteritis. These lesions are described as the enlargement of intestinal villi and their fusion due to massive erosion of the small intestine. In the caecum, there is heavy infiltration of inflammatory cells, with occasional eosinophils (Al-Zarkoushi and Al-Zubaidi, 2021).

The intricate nature of the environment necessitates a similarly complicated immune system that is capable of preventing an exaggerated response against food particles and commensals while also eliminating potential pathogens. One of the mechanisms that support these functions is the intraepithelial lymphocyte (IEL). The IELs are a varied group of immune cells that mainly exist in between intestinal epithelial cells and maintain a close association with these cells (Nazmi *et al*., 2021).

Multi-nucleated giant cells were detected in our study, created through the merging of different types of cells like macrophages, monocytes, epithelioid cells, and others. They are typically large and commonly found in areas of chronic inflammation and other granulomatous conditions (Brodbeck and Anderson, 2009).

An infection of *Eimeria* coccidia can greatly affect the nutritional environment of the chicken's gastrointestinal tract. The presence of the *Eimeria* parasite causes damage to the intestinal lining, impacting both the epithelial cells and the microbial communities within the tract. This damage can lead to an increase in the colonization and growth of harmful pathogens in the chicken's gut, ultimately resulting in a higher mortality rate for chickens. Studies carried out previously have established that the simultaneous presence of *Eimeria* infection can have an impact on the proliferation and colonization of certain bacteria, including *Clostridium perfringens* and *Salmonella enterica* serovar Typhimurium (Collier *et al*., 2008; Macdonald *et al*., 2019).

Eimeria tenella has a life cycle that lasts seven days. During this time, it goes through intracellular development and multiplies by going through distinct intracellular stages that are limited to the cecal epithelium. *E. tenella* first penetrates the epithelial cells that line the crypts of Lieberkhün in the lumen (Del Cacho *et al*., 2014). Schizont maturation occurs when the parasitized host cells separate from the epithelial layer and migrate through the lamina propria deep into the muscularis mucosa, the borderline between the lamina propria and the submucosa (Del Cacho *et al*., 2014).

The asexual cycles of *E. necatrix* happen in the small intestines, while the sexual cycles take place in the ceca. Therefore, the oocysts can exclusively be found in the ceca. Due to poor oocyst production, birds frequently perish before oocysts defecate. Histologically, the extensive tissue damage caused by *E. necatrix* can be detected as the schizonts penetrate deeply into the mucosa and submucosa, thereby destroying blood vessels and smooth muscle. As a result, it becomes difficult for the epithelium to regenerate, and if the birds survive, scar tissue may develop in the intestine (Cervantes *et al.*, 2020). Recently, Almahallawi *et al.* (2024) pointed out that the most notable disease in poultry is coccidiosis, particularly cecal coccidiosis, due to its sudden onset and high death rate. Diagnosis of coccidiosis is generally determined by examining lesions and oocyst secretion. *E. tenella* and *E. necatrix* are responsible for the majority of mortalities in birds affected by coccidiosis, primarily due to cecal lesions.

Conclusion: As far as we know, our study is the first of its kind to investigate the occurrence of *Eimeria* spp. in chickens that are reared in a backyard system in Soran City.

A cross-sectional study was conducted to identify the prevalence and species characterization of coccidiosis. The study found that 41.25% of examined chickens were infected with *Eimeria*. The highest prevalence of the parasite was observed in winter, followed by autumn and spring. The summer season had the lowest number of cases. The confirmation of the genus was obtained by targeting 18S ribosomal RNA using genus-specific primers, resulting in 455 bp of amplified gDNA from oocysts. The four species of *Eimeria* spp. identified were *E. tenella*, *E. necatrix, E. acervulina*, and *E. brunetti*, with *E. tenella* being the most prevalent. The invasion of the parasite to the intestinal layer causes damage and necrosis as well as provokes immune responses that were presented by different inflammatory cells and infiltration mainly lymphocytes, plasma cells, and eosinophils.

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