

Determination of *Eimeria* species in chickens by using traditional methods in Wasit province, Iraq

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Abstract— Coccidiosis is an intestinal parasitic disease due to a parasite of the species *Eimeria*. The present study was carried out to investigate the prevalence of coccidiosis in local breeds of domestic chickens and poultry farms in Wasit region, Iraq. 240 fecal samples were randomly collected from individual backyard scavenging native chickens (n = 100) and commercial poultry farms (n=140) across Wasit province. Sampling was conducted during the period from October to December 2025. The sampling sites included Kut, al-hay and al-Suwaira districts representing the central, southern (central) and northern regions of Wasit. Every fecal samples were tested using the flotation procedure involving sugar and salt solution. The overall infection rate was 73.3% with a prevalence of 64% in backyard chicken and 80 % in poultry farms, based on morphological characteristics of the oocysts, there were six species identified, *Eimeria tenella*, *Eimeria necatrix*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria acervulina* and *Eimeria brunetti*. Morphological characteristics were recorded. *E. mitis* had the highest infection rate (25%), whereas *E. acervulina* and *E. brunetti* had the lowest rates (12.5% each). The study concludes that *Eimeria* species are widely distributed among both backyard and poultry farms chickens in Wasit province. Furthermore, the result indicates that avian coccidiosis remains a significant threat to the commercial poultry population in Iraq; this is the first general report about the infection status of *Eimeria* hens in Wasit, Iraq.

Keywords — Coccidiosis, Diagnosis, *Eimeria* species, Wasit /Iraq.

INTRODUCTION

The poultry industry is an important source of animal protein and employment worldwide (1). Poultry coccidiosis is one of the catastrophic bird diseases which afflict the whole world. This disease is a huge threat to the poultry business since it has a major financial impact as it causes higher mortality, lower weight gain and increased susceptibility to necrotic enteritis (2). Coccidiosis is known as the parasite disease that causes the biggest economic impact on chicken industries globally due to losses in productivity and expenses for treatment or prevention (3). Coccidiosis in chicken may be recognized as clinical, with bloody droppings and increased

mortality, or subclinical, with the presence of the parasite and huge lesions without outward symptoms of the disease leading to considerable financial losses. (4, 5). The birds lose weight rapidly as their immune systems fight infection. Clinical symptoms of the disease are not seen until the second generation of schizonts begins to replicate, grow and mature and release the second generation of merozoites (6-8).

Coccidiosis is the protozoan disease in one form of intracellular parasite which has a major impact on chicken productivity and is seen as economically important (9). Pathogenicity of different *Eimeria* species is moderate to severe, stressing the necessity of knowing the species makeup to implement efficient control and preventative strategies (7). The infection causes diarrhoea, emaciation, enteritis, drooping of wings, poor growth and increased morbidity and mortality (10). Instead, wet litter and excessive stocking density are two bad management practices and confinement rearing methods that enhance the risk of contracting coccidiosis and can worsen its symptoms (10, 11). Species of the genus *Eimeria* which is a member of the phylum *Apicomplexan* and family *Eimeriidae*, cause coccidiosis in poultry. The *Eimeria* genus contains about 1800 species that live in the intestinal mucosa of various mammals and birds, highlighting the parasite's widespread threat (2). *Eimeria* species, which are known to be monoxenous, are host specific, meaning that they only use one host for their entire cycle (12). *Eimeria mitis*, *Eimeria acervulina*, *Eimeria praecox*, *Eimeria maxima*, *Eimeria tenella*, *Eimeria necatrix* and *Eimeria brunetti* are seven coccidian species showing different pathogenicity levels. The most pathogenic *Eimeria* species include *E. necatrix* and *E. tenella*, while *E. acervulina*, *E. brunetti* and *E. maxima* are less pathogenic. On the other hand, *E. mitis* and *E. praecox* are commonly considered to be less pathogenic (13). Effective control methods are important as the main mode of transmission is fecal-oral. Each species of *Eimeria* occupies different regions of the intestinal tract, displaying their particular parasitic behaviour. The direct life cycle of the genus contains both asexual and sexual phases including gametogony (sexual reproduction and gamete formation), schizogony (agamogony/merogony) and sporogony formation (14).

The distribution of these species in the digestive tract, their potential for sickness and death differ (15). *E. tenella* and

E. necatrix are the most pathogenic species. The following species are abundant, mildly to somewhat pathogenic. *E. brunetti*, *E. mivati*, *E. maxima*, and *E. acervuline* are uncommon but dangerous when they occur. *E. hagani*, *E. mitis* and *E. praecox* are non-pathogenic species (16). In poultry records *E. tenella* is the most prevalent coccidia with lesions easily detected and high losses in young broilers and pullet layers (17). It resides in the caecum and causes a dangerous sickness identified by bleeding in digestive tract, significant morbidity and mortality, emaciation and paleness of skin pigmentation, decreased weight gain, and bloody mass in caecum (18). Coccidiosis is diagnosed and *Eimeria* is characterized using a variety of techniques, including the size and shape of *Eimeria* oocysts, distinctive macroscopic lesions at necropsy, sporulation time, and cross-immunization testing (19). Morphological and molecular approaches are necessary for the identification and research of these parasites. Morphological identification involves examining physical traits of *Eimeria*, including the size, shape, and structure of their oocysts (15).

MATERIALS AND METHODS

Sampling collection

Samples collection a total of 240 faecal samples of chickens were randomly collected from individual scavenging native hens from home chicken and poultry farms in Wasit province. The sampling was done between the period months of October and December 2024. The sampling sites were located in the Kut, al-hay, Aziziyah and al-Suwaira districts, representing the southern, central and northern regions of Wasit province. Fresh fecal samples were collected randomly from the environment after excretion and put in different plastic bags and then transported directly to the microbiology laboratory in a cooling bag, University of Wasit, where they were kept in a refrigerator ($> 4-5^{\circ}\text{C}$).

Parasitological techniques

Fecal samples of chicken were examined for the detection of *Eimeria* oocysts by using a sucrose solution (sp.gr. 1.27) and saturated salt solution (sp.gr. 1.20) in the direct flotation method (Soulsby and Helminths. (20)). Three grams of faeces were well hand mixed with 5 ml of sugar-salt flotation solution in a rubber tube with a glass stirring rod. The faeces combination was poured through filter paper in a funnel into a test tube. The test tube was then filled with flotation liquid so that the oocysts float up to the surface. The test tube was securely covered with a cover slip and kept for 10-15 minutes. The floating Oocysts were attached to the coverslip. Then the cover slip was gently removed from the test tube and promptly put on a clean microscope slide. The slide was next examined under light microscope and at 40 X magnification for the presence or absence of oocysts. Morphological identification was based on the size, shape, wall thickness and presence or absence of micropyle. Form, length and thickness of Oocysts. Morphological characterization was made on oocysts (Soulsby and

Helminths (20) using light microscope at 100x and 400x magnifications. and picture by camera mobile (Samsung)

Procedure for sporulation

Unsporulated oocysts from faecal samples were homogenized in water and filtered. The residue was resuspended in 2.5% aqueous potassium dichromate solution. This was to provide appropriate moisture, and destroy other microbes present in the excrement, competing for oxygen and nutrients with the oocysts. The samples were transferred into rubber tubes and maintained at room temperature for sporulation. The samples were kept standing for 24 - 48 h so that unsporulated oocysts might sporulate. Each sample was examined microscopically at different periods to see whether it contained sporulated oocysts. Use ocular Micrometer for oocyst measurements to identify *Eimeria* species, The size and shape of the sporulated oocysts were determined under a light microscope at 40 X magnification according to the description of Conway and McKenzie (21).

STATISTICAL ANALYSIS

The data collected for the study were input in Microsoft Excel spreadsheet and analyzed using the SPSS Statistical Package (Version 25.0). The incidence of theileriosis was determined monthly as the ratio of infected animals to the total cases. One-way ANOVA and chi-square tests were used analysis the differences in prevalence between sheep and cattle. Statistical significance was $P < 0.05$ set at.

RESULT AND DISCUSSION

Microscopic examination

Eimeria oocysts were detected in 73.3% (176/240) of the microscopic examination of faeces samples. The isolated *Eimeria* species were identified and differentiated according to the morphometric and biological criteria summarized in Table 1, identified as The oocysts appeared spherical to ovoid in shape with characteristic morphological features, under the microscope on prepare slides, in Fig-ure-1.

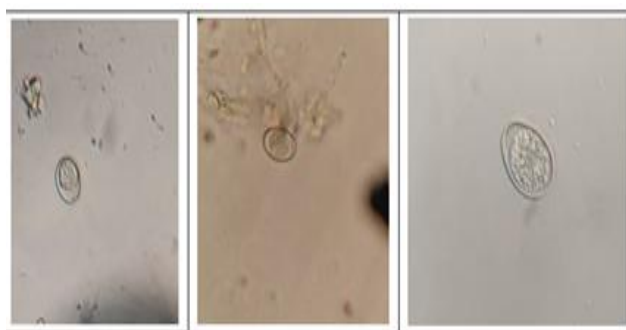


Figure 1. Microscopic appearance of *Eimeria* oocysts under light microscope (40x)

In the current study the length, width and shape of the oocyst were indicative for the identification of *Eimeria* species. Sporulation duration, gross lesion and oocyst morphology may also assist in this process. The majority of the oocysts of *Eimeria* were ovoid in shape. This is in agreement with the findings of Soulsby (22) who found the size of *E. tenella* to be 19.5-26 by 16.5-22.8 μm (23,24) 00, *E. acervulina* 15.7-20.3

by 14.5-18.6 μm , *E. maxima* 27.9-34.5 by 18.6-26.4 μm and *E. brunetti* 20.6-26.3 by 16.9-21.6 μm .

In our study poultry farms, exhibited a significantly higher infection rate (80%, 112/140) than backyard chicken (64 %, 64/100) table(2) in a number of areas of Wasit province from which samples were taken as Kut, Hay and Suwaira a backyard chicken (18.75%, 56.25%, 25%) and in poultry farms (14.29%, 0 , 85.71 %) respectively

Statistical analysis revealed a highly significant spatial distribution of parasitic infections across the studied districts ($P < 0.001$). The calculated P-value confirms that the variation in prevalence rates notably particularly the high prevalence in Suwaira poultry farms and Hay backyard chickens is statistically significant. This allows for the rejection of the null hypothesis and confirms that geographical location and management systems are decisive factors in the epidemiology of the *Eimeria* within Wasit province as in figure 2.

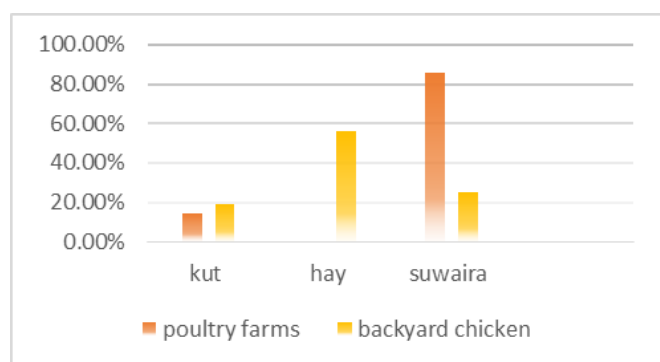


Figure 2. The figure shows the spread of *Eimeria* sp. in the cities of Wasit.

Our data about the prevalence of *Eimeria* in domestic chickens are similar to those of Rashid and Shnawa (25), who reported the occurrence of coccidiosis in backyard chickens in Erbil, Iraq. Out of 400 investigated intestinal scraping and fecal samples, 4 species of *Eimeria* were detected in 165 (41.25%) samples. Rates of coccidiosis in native breed chickens in Tunisia and Nigeria were reported to be 31.8% and 35.5%, respectively (26,27) . Coccidiosis was found in 54.3%, 64%, 53.6%, and 21.24% of poultry in Turkey (28) , Ethiopia and Egypt respectively (28). The differences in prevalence rates may be due to a variety of factors, such as breeding conditions, the location of the study, disease management strategies,

Table 1. Morphological characterisation of *Eimeria* spp. isolated from chicken

<i>Eimeria</i>	Oocyst size (μm)	Mean of Width and length (μm)	Sporulation time (h.)	Morphology
<i>E. mitis</i>	14.7×15	11.5-17.5×12.5-18	18	"Thin wall, sub spherical"
<i>E. maxima</i>	22.3 × 29.9	23-30.5 × 18-24	30	"Large ovoid, thick wall, double-contoured lines, yellow-low brown"

<i>E. tenella</i>	15 ×19	20× 25	20	Ovoid, thin smooth wall
<i>E. necatrix</i>	16.7×19.1	"12.5-18 × 22.5-14"	20	oblong ovoid, thin wall
<i>E. acervulina</i>	15.23×19.2	17.9-21.5 × 14.2-16	18	"Thin smooth wall. Ovoid. Narrow anterior end."
<i>E. brunetti</i>	19.74×23.4	21-28.2 × 18.5-22	20	thick brown ovoid wall

climate and environmental changes in the area, the season when the samples were collected, and the differences in the number of samples and the age of the hens used (29).

It was also said that the *Eimeria* infection rate is quite high in field-raised hens and is related to a number of risk factors including poor management, humidity, poor ventilation systems and inadequate biosecurity procedures on the farms. The incidence of *Eimeria* spp. was variable in different nations, diagnostic procedures, and host situations such as ages, control treatments, and demographics. The findings were in agreement with Ismael *et al.* (30).

The results showed that coccidiosis was highly prevalent on barbecue farms in Duhok Province 60% , Iraq based on collection of 600 hens with ceca. Coccidiosis in chickens of Al-Diwaniyah City, Iraq, 467 hens (190 layers and 277 broilers) were randomly collected from intestinal contents and fecal samples from farms and shops nearby. Macroscopic and microscopic analysis of the samples revealed an incidence of droppings of 30.68% $n = 85/277$, in addition, microscopic examination of samples revealed that the incidence of droppings was 30.68% ($n = 85/277$) for both macroscopic and microscopic studies (31).

In Rahman *et al.* (32), the authors observed that the infection rates of hens fed with commercial feed 7.88% were significantly higher than that of birds fed with local feed 3.99%

. Infection rates were higher in intensive rearing systems 15.27% than in free-ranging systems. .78% in Jordan and 29% in Romania (33) in agreement with the finding of Al-Natour *et al.* (34). This incidence rates were 38% in Northeast area of Iran, 55.96 Northwest region and 64 (35).

In our study, the *Eimeria* species identified in samples from different districts of Wasit were (*E. mitis*, *E. maxima*, *E. tenella*, *E. acervulina*, , *E. necatrix*, and *E. brunetti*). The calculated P-value of 0.043 indicates that the observed variations in species distribution are statistically significant (table 2).

Table 2. distribution and prevalence of *Eimeria* species (n: 160) in Wasit

Species	Positive samples	Prevalence among <i>Eimeria</i> species (%)
<i>E. mitis</i>	40	25%
<i>E. maxima</i>	32	20%
<i>E. tenella</i>	24	15%
<i>E. necatrix</i>	24	15%
<i>E. acervulina</i>	20	12.5%
<i>E. brunetti</i>	20	12.5%
	160	100%
$\chi^2 = 11.44$		P = 0.043

The result was agreement with many study Rahman *et al.*, (32) who reported Six species have been identified, including *E. acervulina*, *E. tenella*, *E. mitis*, *E. brunetti*, *E. necatrix*, and *E. maxima*. Morphometric examination of sporulated oocysts to identify the species of *Eimeria* in Erbil, Iraq. The investigation revealed four species of *Eimeria*: *Eimeria tenella* 25%, *E. necatrix* 20%, *E. acervulina* 15%, and *E. brunetti* 7% (25). the faecal testing revealed 32.6% of the total samples were positive for *Eimeria* oocysts, which were divided into six species including *E. brunetti*, *E. mitis*, *E. Maxima*, *E. acervulina*, *E. necatrix*, *E. tenella* with infection rates 57.5%, 44.2%, 42.1%, 26.5%, 20.4% correspondingly (36).

The biggest diversity of *Eimeria* species was found in broilers, which included *E. acervulina* 44.45% and less common species like *E. mitis* and *E. necatrix* 7.41% each, This suggests that broilers are susceptible to various infections, which is probably caused by their intense farming settings (37). Moreover, Mares *et al.* (38) reported 10.84%, 5.84%, 4.16%, 2.5%, and 1.66% of *Eimeria* spp., *E. tenella*, *E. acervulina*, *E. necatrix*, *E. praecox* and *E. maxima* infections, respectively. *E. acervulina* was also the most prevalent species in Egypt, followed by, *E. necatrix*, *E. tenella* and *E. mitis*. Based on their research, coccidiosis is a severe parasitic disease that has a great impact on the productivity of chickens (39).

CONCLUSION

To our knowledge, this is the first comprehensive evaluation of the prevalence of *Eimeria* infection across different breeds of chickens in Wasit. Overall prevalence of *Eimeria* infection was 73.3%. The most prevalent *Eimeria* species identified were *E. mitis*, *E. maxima* and *E. acervulina* followed by *E. brunetti*, *E. necatrix*, and *E. tenella*. Management systems played an important role in the prevalence of infection. The highest risk factor for coccidiosis was intensive rearing and the lowest prevalence was in free-range systems. Also, chickens fed commercial diets were more susceptible to sickness than those fed locally sourced feed. These results underline the necessity for focused control measures, especially in intensive agricultural systems and high-risk breeds. Good management techniques, such as good cleanliness and robust biosecurity controls, are needed to control and prevent disease. The future research should focus on sustainable approaches for coccidiosis prevention in chicken production, with particular emphasis on the resistance mechanisms of naturally resistant breeds..

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Conflict of Interest

The authors declare no conflict of interest.

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