



## Traditional Diagnosis of *Eimeria* spp. in Fallow Deer at Middle Parts of Iraq

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### A B S T R A C T

*Eimeria* is an apicomplex protozoan which affects many species of birds, domestic and wild animals including deer. This is the first study in Iraq that was designed for diagnosis *Eimeria* spp. in deer using traditional methods. Eighty fecal samples of Fallow deer (*Damadama*) were collected in different provinces in the middle part of Iraq during December 2018 to the end of September 2019. Samples were examined by direct smear and flotation with Sheather's solution. The total infection rate with *Eimeria* spp. was 70% (56/80), in which higher infection rate was observed in female deer (78%, 39/50). The results also showed that Fawn at age <3-11 months recorded higher rate of infection (86.3%, 19/22). *Eimeria* infection was more prevalent in Karbala and Baghdad provinces where recorded (73.3% and 72%) respectively, sharp increase of infection recorded during March (87.5%, 7/8). In addition, four species of *Eimeria* (*E. crandalis*, *E. intricate*, *E. parva*, and *E. sordida*) were detected in Iraqi deer according to morphological characterization of oocysts. This study highlighted the distribution of some *Eimeria* species in deer and revealed the effect of some epidemiological factors on prevalence in different middle Iraq provinces.

**Keywords:** traditional, diagnosis, *Eimeria*, fallow, deer

### INTRODUCTION

The genus *Eimeria* (Apicomplexa: Eimeriidae) is a species group of single-celled parasitic organisms that are obligatory intracellular protozoan parasite (1, 2). These parasites are common in most domestic and wild ruminants, such as cattle (3), sheep and goats (4, 5) and deer (6) including fallow deer (7).

*Eimeria* spp. can be transmitted by fecal-oral route. The source of infection is usually by asymptomatic carrier adult animals that act as a source for spreading infection through contaminating water and feed (8). The appearance of clinical signs depends on the number of ingested

sporulation oocysts (9). This coccidiosis can be found probably in all ages of animals and can be a significant problem in the younger one (10).

*Eimeria* 's taxonomy relied on the morphological characteristics of the sporulated and non-sporulated oocysts. The basic structure component of the oocyst wall is the same in all *Eimeria* species and the structure of the sporozoites includes refractile bodies, the nucleus, and striations (11).

In Iraq, rare available studies were recorded the *Eimeria* detect in deer. Therefore, this study was designed to investigate the rate of infection with *Eimeria* spp. in deer in middle areas of Iraq; and to detect types of *Eimeria* spp. in

Iraqi deer according to the morphological characterization of oocysts.

## MATERIALS AND METHODS

### Sampling

All procedures conducted in this study was reviewed and approved by the scientific committee in the College of Veterinary Medicine, University of Baghdad in accordance with the ethical standards of animal welfare.

A total of 10-15 grams fecal samples were collected from rectum of 80 Fallow deer (30 males, 50 females) with different ages (<3-11 months, <11-20 months and <2 years) from different provinces in the middle areas of Iraq (Baghdad (25), Al- Najaf (15), Babylon (25) and Karbala (15) during December 2018, to end of September 2019.

Fecal samples were collected in sterile tightly stopper plastic containers. All information included age sex and date of sampling were given serial numbers; and change gloves after collecting samples as a protective step were done too. The samples were dispatched by cooling box to the laboratory of Parasitology, College of Veterinary Medicine/ University of Baghdad for diagnosis using traditional methods (direct smear and flotation with Sheather's solution).

### Direct Wet Smear

One gram of each fecal samples was placed onto the glass slide after one drop of normal saline was added on it and mixed with a wood stick; then a coverslip was added on each slide. The slides have been examined under the light microscope at 10x and 40x magnification powers (12).

### Flotation Method

The flotation method as described by (13) based on the use of Sheather's solution. The steps of the method were by examining a batch of 2-4 g of feces with 10 mL of distilled water. Then to extract big fragments, the mixture of feces was washed out with sieve sized of forty angles. The filtrates were placed in sterile plastic tubes and centrifuged at 1000 rpm for 3 min then the supernatant was discarded. 5 mL volume of sugar solution was applied for precipitation using wooden sticks; then the latter mixed properly and centrifuged at 1000 rpm for 2 min. All plastic test tubes were put on hold to fill the tanks letting the Pipette to drop sugar solution vertically. Finally, the glass cover slide was then put on the tubes for 10-15 min.

To scan the *Eimeria* oocysts, the glass cover slide was carefully lifted and placed under a microscope at magnification strength between 10x and 40x (14).

### Oocyst Measurement

The calibration of a microscope for measuring oocyst was provided by (14).

### Statistical Analysis

Statistical analysis was performed using SAS (version 9.1). The infection rates were compared using Chi-square test (15).

## RESULTS

The total rate of infection with *Eimeria* spp. in Fallow deer was 70% (56/80) when traditional method (direct smear and flotation with sheather's solution) of middle areas of Iraq was used.

### Rate of Infection Based on Sex

A significant difference ( $P < 0.01$ ) was reported between male and female deer to their ratio of infection with *Eimeria* spp. in which it was 78% (39/50) in female while male had 56.67 % (17/30) (Table 1).

**Table 1.** Rate of infection by *Eimeria* spp. in fallown deers (*Dama dama*) based on sex

Sex	Total No.	Positive		
		No.	%	Statistics
Male	30	17	56.67	P<0.043
Female	50	39	78	
Total	80	56	70	

$\chi^2 = 8.106, P \leq 0.05$

### Rate of Infection Based on Age

Higher rate of infection with *Eimeria* spp. recorded 86.36% (19/22) in <3-11 months age group while it showed less ratio in > 2 years of age 62.5% (20/32) with significant difference  $P < 0.05$  (Table 2).

**Table 2.** Rate of infection by *Eimeria* spp. in fallown deers (*Dama dama*) based on age

Age	Total No.	Positive		
		No.	%	Statistics
<3-11months	22	19	86.36	P<0.14
11-20 months	26	17	65.38	
>2 year	32	20	62.5	
Total	80	56	70	

$\chi^2 = 8.04, P \leq 0.05$

### Rate of Infection Based on Area of Study

Karbala and Baghdad recorded higher rate of infection 73.33% (11/16) and 72% (18/25) respectively without significant differences  $P > 0.05$  (Table 3).

**Table 3.** Rate of infection by *Eimeria* spp. in fallown deers (*Dama dama*) based on area of study

Area	Total No.	Positive		Statistics
		No.	%	
Baghdad	25	18	72	P<0.96
Karbala	15	11	73.33	
Al-Najaf	15	10	66.67	
Babylon	25	17	68	
Total	80	56	70	

$\chi^2=6.506$ ,  $P\leq 0.05$

### Rate of Infection Based on Month of Study

Non-Significant variation ( $P>0.05$ ) was recorded among the months of the study. March recorded higher rate of infection (87.5 %, 7/8) while the lower rate was recorded in December (44.44%, 4/9) (Table 4).

**Table 4.** Rate of infection by *Eimeria* spp. in fallown deers (*Dama dama*) based on month of study

Months	Total No.	Positive		Statistics
		No.	%	
December	9	4	44.44	P<0.77
January	10	6	60	
February	6	4	66.67	
March	8	7	87.5	
April	10	8	80.0	
May	4	3	75.0	
Jun	9	7	77.78	
July	8	6	75.0	
August	8	6	75.0	
September	8	5	62.5	
Total	80	56	70.0	

$\chi^2=8.777$ ,  $P\leq 0.05$

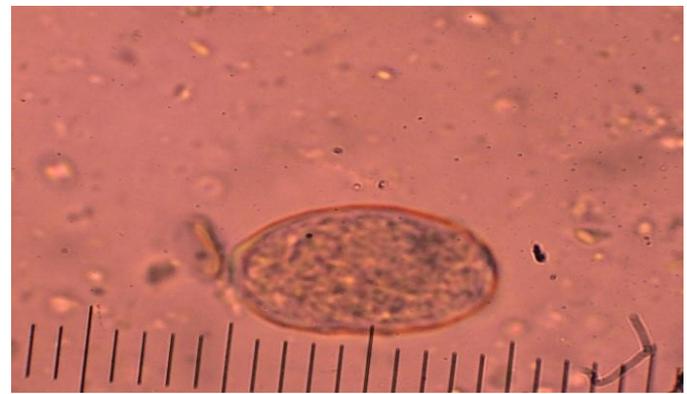
### Oocysts of *Eimeria* spp in Deer

Four species of *Eimeria* (*E. crandalis*, *E. intricata*, *E. parva*, and *E. sordida*) were detected in this study by the traditional methods (direct wet smear and floatation with Sheather's solution) according to the morphological characterizations and measurements of oocysts.

#### *Eimeria crandalis*

Non sporulated oocysts had broad ellipsoidal to spherical shape with average size  $28\times 20\pm 2$   $\mu\text{m}$ , with smooth wall, yellow color, micropyle was presented however, polar cap might be absent (Figure 1).

Sporulated oocysts had an ellipsoidal in shape with 4 sporocysts each one of them had two sporozoites (Figure 2).

**Figure 1.** Non sporulated *Eimeria crandalis* oocyst is seen using the method of floatation with Sheather's solution (40 $\times$ )**Figure 1.** Non sporulated *Eimeria intricata* oocyst is seen using the method of floatation with Sheather's solution (40 $\times$ )

#### *Eimeria intricata*

Non sporulated oocysts had spherical shape with average size  $50\times 37.5\pm 2$   $\mu\text{m}$ , with two layers of irregular granular form thick and striated wall had brown color, micropyle and distinct polar cap (Figure 3).

Sporulated oocysts appeared as spherical shape with four sporocysts each one contained two sporozoites (Figure 4).

**Figure 3.** Non sporulated *Eimeria intricata* oocyst is seen using the method of floatation with Sheather's solution (40 $\times$ )



Figure 4. Sporulated *Eimeria intricata* oocyst by is seen using method of floatation with Sheather's solution (40×)

### *Eimeria parva*

No sporulated oocysts had spherical to sub-spherical shape with an average size of  $15 \times 13 \mu\text{m}$  and smooth colorless surface; absence of polar cap and micropyl had also features of them (Figure 5).

Sporulated oocysts appeared as spherical to sub spherical shape with 4 sporocysts each one of them had two sporozoites (Figure 6).

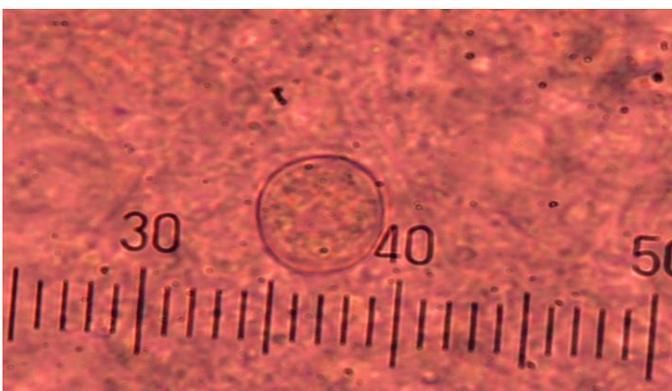


Figure 5. Non sporulated *Eimeria parva* oocyst is seen by floatation with Sheather's solution method (40×)

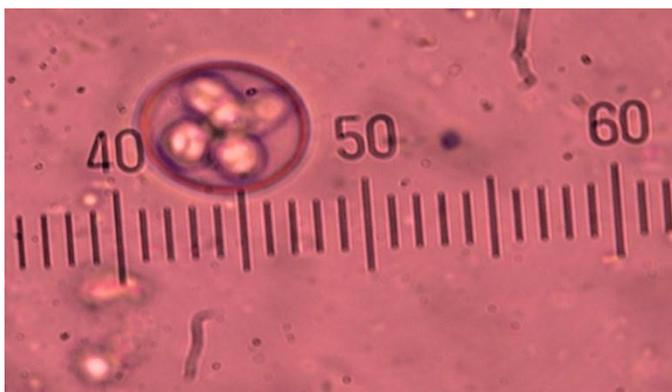


Figure 6. Sporulated *Eimeria parva* oocyst is seen by floatation with Sheather's solution method (40×)

### *Eimeria sordida*

Non sporulated oocyst appeared as spherical to ellipsoidal shape with smooth colorless, non-clear or absent polar cap and inconspicuous micropyle having an average size of  $36 \times 28 \pm 3 \mu\text{m}$  (Figure 7).

Sporulated oocysts appeared as spherical to ellipsoidal shape with four sporocysts each of them had two sporozoites (Figure 8).

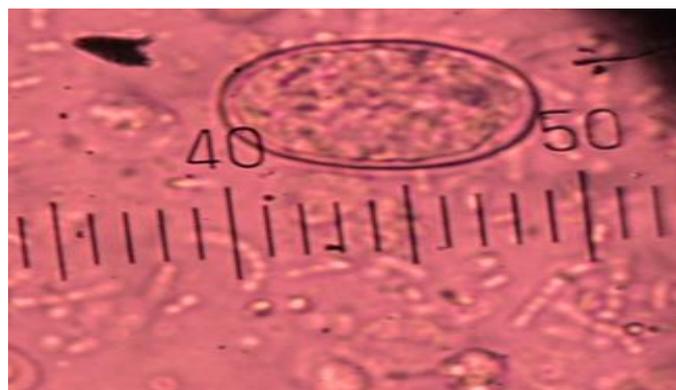


Figure 7. Non sporulated *Eimeria sordida* oocyst is seen using the method of floatation with Sheather's solution (40×)

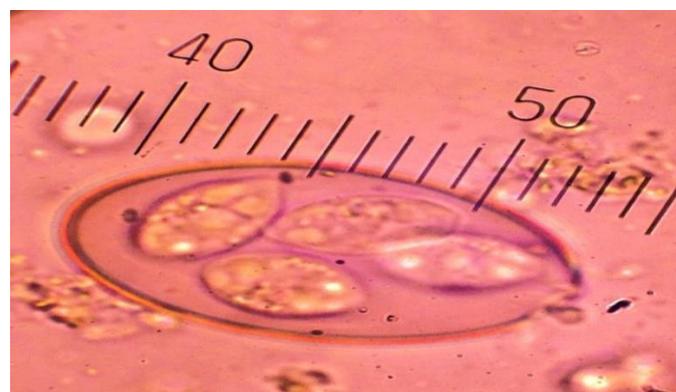


Figure 8. Sporulated *Eimeria sordida* oocyst using method of floatation with sheathers solution (40×)

## DISCUSSION

Total rate of infection with *Eimeria spp* in deer by traditional methods (Direct wet smear and Flotation Sheather's solution) in middle areas of Iraq was 70%. The results of deer were higher than 39, 30% in Bosnia and Herzegovina by others (16).

In wild deer (17), it was recorded a ratio of 81.25% and prevalence for *Eimeria* with micropyle and *Eimeria* without micropyle was 77.78% and 86.67% of equal prevalence for both *Eimeria* with micropyle and without micropyle. *Eimeria spp.* was also recorded in conservation area of Nepal.

Concurring to a few points about the variability in prevalence and distribution of coccidiosis may be due to variations in management and hygienic conditions, temperature, agroecology, environmental, weather conditions, host immune status, sample size, sampling period and breed susceptibility to coccidia in different areas (18). Dissemination of the *Eimeria* oocysts is feasible, particularly in highly productive crowded farms, and those have a highly infected rate readily introduced (19).

According to sex, males and females of fallow deer recorded 56.6% and 78% rate of infection with *Eimeria*, respectively. Walaa et al found that sex had an influence on the prevalence of infection with coccidiosis in adult female in small ruminants (20). The results of same study were in accordance with (21, 22) through which it was revealed that females were more susceptible than males, and the latter showed to be in consistent with the findings of (23) in wild ruminants. The higher rate of infection in females were recorded in compared to males due to higher stressful conditions experienced by female animals especially during pregnancy, delivery, breast feeding and presenting large numbers of females for reproductive and economic purposes in the livestock (23).

In relation to age groups, Fawn recorded higher infection rates with *Eimeria* spp. (86.36%) at the age group (<3-11 months). That was in accordance with others (24) in which they indicated that newborn and less than 1-year old of small ruminants had greater infection rate with *Eimeria* spp. as comparing with older animals. Many studies also showed the relationships between highest infection rate and the ages of ruminants (25, 26).

The young ruminants are more susceptible to infection than older one due to their great susceptibility to infection, the breeding and overcrowding system observed in the different properties, immature development of the immune system of young ruminants in comparison to older. The young ruminant's immune system is still unaware about the invading *Eimeria* parasite because of lack of previous exposure while adult animals had previous multiple exposure to *Eimeria* parasite. Multiple exposures of low dose infection are one of the most important factors that make the animal highly immunized to a specific infection (25).

Karbala province showed a slight increase infection rate with *Eimeria* spp in Fallow deer (73.3%). The differences could be due to living conditions of captivity which made the animals more susceptible to parasitic infection in comparison to the range conditions. Another possibility of parasite transmission was where animals are moved from one enclosure to another without proper parasites precautions. The non-healthy regime of some worker within zoos may increase incidence of infection (26,27).

According to months, March revealed the highest rate of infection with *Eimeria* spp (87.5%) than other months of study. High prevalence of infection during the spring season

may be attributed to climatic conditions that are more conducive to sporulation and coccidian oocyst survival. The result indicated that the rate of infection among deer pastures was high. The reasons for seasonal variation in the rate of infection may be due to variation in temperature, raining, moisture which may facilitate the maturation, shedding, and sporulation of oocysts (28).

The direct examination and flotation method gave the same results regarding diagnosis of *Eimeria* infection, and the only superior advantage of flotation method is that it provided better morphologic parameters to confirm the already obtained diagnosis by direct method.

The studies of (29) and (30) suggested that *Eimeria* were consistent with the basic structure of the oocyst wall. The authors have convincingly shown in both experiments that the oocyst wall consists of two distinct layers, surrounded by an outer membrane, called the outer veil which is usually absent in mature oocysts isolated from feces. The bilayer exhibit is at chances with multiple prior thoughts on which in their definitions called oocysts with single layered walls. The micropyle can be described as discontinuity in one of the layers of the oocyst wall. This feature can be seen in the interior layer as described by (31).

The presence or absence of a micropylar cap, the diameters of the oocyst and the form of the oocysts are valid criteria for differentiating *Eimeria* species (32). Four species of *Eimeria* were detected in this study according to the morphological characterization and measurements of oocyst. Female recorded higher rate of infection than male and fawn deer was more susceptible to infection with *Eimeria* than adult. Finally, infection rate in middle provinces of Iraq recorded significant differences with higher increase during March.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## التشخيص التقليدي لطيفلي الأميريا في غزال الايائل الأديمي في مناطق وسط العراق

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## الخلاصة

طيفلي الأميريا هو أحد الاوالي ذات المعقد الفموي والذي يصيب انواع كثيرة من الطيور والحيوانات البرية والاليفة ومن ضمنها الغزلان. تعتبر هذه الدراسة الاولى في العراق التي تم تصميمها لتشخيص انواع طيفلي الأميريا في الغزلان بالطرق التقليدية. تم فحص ثمانين عينة برازية من غزلان الايائل الأديمي بالطرق التقليدية (مسحات البراز المباشر والتطويق) من مختلف محافظات وسط العراق خلال الفترة من كانون الاول 2018، حتى نهاية ايلول 2019. معدل الاصابة الكلية بأنواع طيفلي الأميريا 70% (80/56)، ولوحظت اعلى نسبة في الاناث 78% (50/39)، كذلك اظهرت الدراسة ان الغزلان الصغيرة ذات الفئة العمرية < 3-11 شهر سجلت اعلى نسبة اصابة 86.3% (22/19). كانت اصابات الطيفلي أكثر انتشارا في محافظتي كربلاء وبغداد وقد سجلت 73.3% (15/11) و 72% (26/18) على التوالي. كما اظهر شهر اذار زيادة حادة في نسبة الاصابة وبلغت 87.5% (8/7). تم الكشف عن اربعة انواع من طيفلي الأميريا في الغزلان في وسط العراق وفقا للتوصيف الشكلي للكيس البيضنة وعلى النحو التالي (*E. crandalis*, *E. intricata*, *E. parva*, and *E. sordida*) سلطت هذه الدراسة الضوء على انتشار بعض انواع طيفلي الأميريا في الغزلان باعتبارها مضافات جديدة وسجلت تأثير بعض العوامل الوبائية على الانتشار في محافظات وسط العراق.

الكلمات المفتاحية: الأميريا، غزال، الايائل، الأديمي، طيفلي