



TRADITIONAL DETECTION OF *EIMERIA* SPP. IN BUFFALOES IN AL-QADISSIYHA PROVINCE, IRAQ

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Abstract

Eimeria is an apicomplexan protozoa which affect many species of wild and domestic animals and birds including buffaloes. The study was designed for investigation *Eimeria* spp. in buffaloes by traditional methods. One hundred Eighty fecal samples of buffaloes were examined (direct smear and flotation with sheather's solution) from different Areas in Al – Qadisiyha province of Iraq. During December 2018, to end of August 2019. A total infection rate with *Eimeria* spp was 53.8% (97/180). higher rate recorded by female 58.3% (70/120) and age record group at (1<3year) recorded higher rate of infection 60% (30/50) . *Eimeria* infection was prevalent in Al-Sadeer area recorded 58.3% (35/60) and the infection showing sharp increasing in March 85% (17/20). Four species of *Eimeria* were detected in Iraqi buffaloes according to morphological characterization of oocysts as following (*E. bovis*, *E. zurnii*, *E. subsphrica* and *E.cylindrica*). The sporulation time different according to *Eimeria* spp. average between 3-5 days.

Introduction

The genus *Eimeria* (Apicomplexa: Eimeriidae) is a species group of single-celled parasitic organisms the obligatory intracellular protozoan parasite (Shirley *et al.*, 2005, Yakhchalil and Rezaei, 2010). It is well documented that the Coccidiosis is frequently encountered in goats, sheep and cattle but it is less frequent in horses (Cooke *et al.*, 2013). *Eimeria* spp. Transmitted by fecal-oral, The source of infection is usually by asymptomatic carrier adult animals, These carries act as a source for spreading infection through water and feeding sources (Ocal *et al.*, 2007). Coccidiosis is a generalized term used for a group of sporozoa in the Eimeriidae family that are commonly parasitic in the host intestinal tract, but occasionally also in the liver and kidneys, including three genera *Eimeria*, *Isospora* and *Cystoisospora*, coccidia infection causing enteritis in all species (Coetzer and Justin, 2004). The presence of clinical signs depends on the amount of sporule oocysts ingested. (Peek, 2010). This disease has probably been found in all animal ages and can be a major problem in the younger one. (Urquhart *et al.*, 1996). *Eimeria*'s taxonomy relied on the morphological characteristics of the oocysts and speculated oocysts (Ogedengbe *et al.*, 2015). The study designed to reveal the rate of infection with *Eimeria* spp. in buffaloes in Al –Qadisiyha province of Iraq according to morphological

characterization of oocysts.

Materials and Methods

A total of (10-15) grams fecal samples collected from (180) (60 male, 120 female) with different ages, from some areas from Al –Qadisiyha of Iraq (Al-Sadeer (60), Al- Sanyia (40), Al-Dagarah (40) and EfK (40)) samples during December 2018, to end of August 2019. Fecal samples collected in clean plastic container and were tightly closed, given sequential numbers, with taking off protective measure such as wearing disposable gloves. All information included age, sex, and date of sampling. The samples were transported by cooling box to a laboratory of Parasitology/College of Veterinary Medicine- University of Baghdad/ Iraq for traditional methods (direct smear and flotation with sheather's solution).

Direct Wet Smear

2-4 gm of fecal samples were put on a glass slide. A coverslip was applied after addition of one drop of normal saline and mixed with a wood stick. The examination was done with light microscopy at 10X and 40X magnification powers (Cole, 1986).

Flotation method

The flotation method based on the use of Sheather's Solution as following:

1. A specimen of 2-4 g of feces has been mixed with low amounts (10 ml) of distilled water.
2. The feces solution was placed in a sieve of 40 anges to remove large particles.
3. The filters were accumulated through sterile plastic tubes and put now a separator at 1000 rpm for three min. there sup. Then rejected.
4. A small amount of Sheather 's Solution was used to precipitate and well blended with wooden sticks. It was then placed in a centrifuge at 1000 RPM for 2 minutes.
5. Both plastic test tubes were put on hold and upright, and drops of Sheather 's Solution were applied to the pipette to fill the tubes. Then the glass cover slide was put on the end of the tubes for 10-15 minutes.
6. The glass-covered slide was gently raised and positioned under a 10X and 40X magnification microscope to search for the Eimeria oocysts. (Al-Kaabi, 2009).

Measuring of Oocysts:

The calibration of oocysts was provided by Conway and Mckenzie, 2007 as following:

- 1- Using the low power found in the compound microscope, the stage micrometer lines to brought into focus and attuned the zero line of the stage micrometer to match with the zero line of the ocular micrometer.
- 2- Further line on the ocular micrometer which exactly coincides with a second line on the stage scale was found.
- 3- The number of spaces between the two lines was counted using the ocular scale and divided this number into the number of microns represented between the two lines on the stage [number of small spaces X10microns].

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Results

The total rate of infection with *Eimeria* spp. in buffaloes by traditional methods (direct smear and flotation with sheather's solution) in Al- Qadisiyha province in Iraq 43.8% (97/180).

Rate of infection with *Eimeria* spp. in buffaloes according to sex

According to sex, total number of female animals

was 120 of which 70 (58.3%) were infected as proven by direct and flotation methods, whereas the total number of male animals was 60 of which 27 (45%) animals had infection as it was proven by direct and flotation method without significant differences ($p>0.05$) table 1.

Table 1: Rate of infection with *Eimeria* spp. in buffaloes according to sex.

Sex	No. of Samples examined	No. of Positive	Infection Rate %
Male	60	27	45
Female	120	70	58.3
Total	180	97	53.8
X ²			2.862
P value			0.091 (NS)

X² chi-square, NS: No significant difference ($P>0.05$).

Rate of infection with *Eimeria* spp. In buffaloes according to age groups

The results showed no significant difference ($p>0.05$) in the prevalence rates among different age groups, the animals included in the present study were classified into three age group, less than one year, one year up to less than three years and more than or equal to three years as shown in table (4-4). The first group, less than one year of age, involved 50 animal of which 30 animal proved to be infected by direct and flotation method and the rate of infection in this group of age was 60 %. The second group, one year up to < three years, involved 70 animals of which 38% proved to carry infection by direct and flotation method and the rate of infection was 54.2%. The third group, more than or equal to three years, was composed of 60 animal of which 31 were infected and the rate of infection was 51.6%. table 2.

Rate of infection with *Eimeria* spp. In buffaloes according to the areas of study:

Table 2: Rate of infection with *Eimeria* spp. In buffaloes according to age groups.

Sex	No. of Samples examined	No. of Positive	Infection Rate %
1<year	50	30	60
1 to<3years	70	38	54.2
≥3Years	60	31	51.6
Total	180	97	53.8
X ²			0.789
P value			0.674 (NS)

X² chi-square, NS: No significant difference ($P>0.05$)

The rate of infection in each district, total Cases collected from AL-Sadeer district were 60 of which 35 (58.3%) were infected. Total Cases collected from AL-Dagarha district were 40 of which 23 (57.5%) were

infected. Total Cases collected from Efak district were 40 of which 20 (50%) were infected. Total Cases collected from AL-Sanyia district were 40 of which 19 (47.5%) were infected, the results indicated that no significant differences ($p > 0.05$) in the prevalence rate in these different regions table 3.

Rate of infection of *Eimeria* spp. in buffaloes according to months of study

Table 3: Infection rate of *Eimeria* spp. in buffaloes according to areas of study.

Sex	No. of Samples examined	No. of Positive	Infection Rate %
AL-Sadeer	60	35	58.3
AL-Dagarha	40	23	57.5
Efak	40	20	50
AL-Sanyia	40	19	47.5
Total	180	97	53.8
X ²	1.587		
P value	0.662 (NS)		

X² chi-square, NS: No significant difference ($P > 0.05$).

The results showed that the highest prevalence rate recorded in March 85%, while the results were convergent during the months of May and June, 50%, 50%, respectively. The lowest prevalence rate recorded during January 35%, these results showed significant difference ($p \leq 0.05$) table 4.

***Eimeria* spp. Oocysts in buffaloes detection in study**

In this study, four species of *Eimeria* were detected by conventional methods (direct wet and float with Sheather 's solution) and by morphological characterization and measurement of oocysts collected.:-

Table 4: Rate of infection with *Eimeria* spp. in buffaloes according to months of study.

Month	No. of Samples examined	No. of Positive	Infection Rate %
December	20	8	40
January	20	7	35
February	20	9	45
March	20	17	85
April	20	14	70
May	20	11	55
June	20	11	55
July	20	9	45
August	20	11	55
Total	180	97	53.8
X ²	15.606		
P value	0.048 (S)		

X² chi-square, S: Significant difference ($P < 0.05$)

(*E. bovis*, *E. cylindrica*, *E. subsphrica* and *E.zurenii*).

Eimeria bovis

-Non sporulated oocysts:- Characterized by broad ellipsoidal to spherical shape and has smooth wall, pale-yellowish in color, medium size ($28 \times 2 \pm 20$) μm , micropyle present, polar cap may be absent Fig. 1.

-Sporulated oocysts:- appeared broad ellipsoidal in shape with 4 sporocysts each one contain 2 sporozoite Fig. 2.

Eimeria subsphrica

-Non sporulated oocysts:- Ellipsoidal shape very



Fig. 1: Non sporulated *Eimeria bovis* oocysts by floatation with Sheather 's solution (X40).

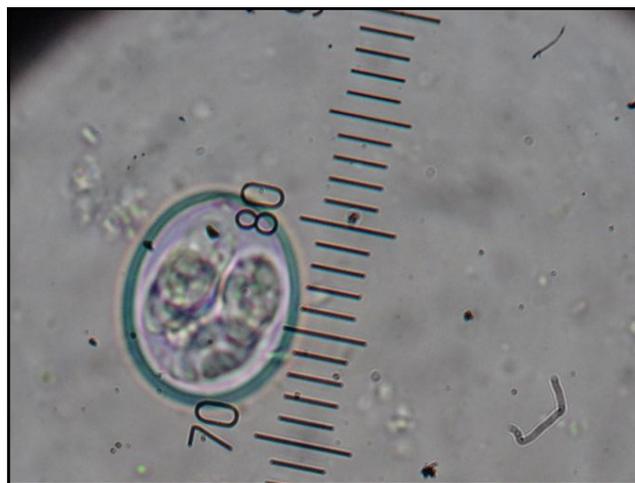


Fig. 2: Sporulated *Eimeria bovis* oocysts by floatation with Sheather 's solution (X40).

smooth wall colorless, polar cap absent, micropyle absent, medium size (12.5×10) μm Fig. 3.

-Sporulated oocysts:- appeared ellipsoidal shape with 4 sporocysts each one contain 2 sporozoites Fig. 4.

Eimeria cylindrica



Fig. 3: Non sporulated *Eimeria subssphrica* oocysts by floatation with with Sheather’s solution (X40).

-Non sporulated oocysts:- Ellipsoidal shape smooth wall colorless, polar cap indistinct or absent, micropyle



Fig. 4: Sporulated *Eimeria subssphrica* oocysts by floatation with Sheather’s solution (X40).

inconspicuous, medium size (20x12.5)µm Fig. 5.

-Sporulated oocysts:- appeared ellipsoidal shape with 4 sporocysts each one contain 2 sporozites Fig. 6.

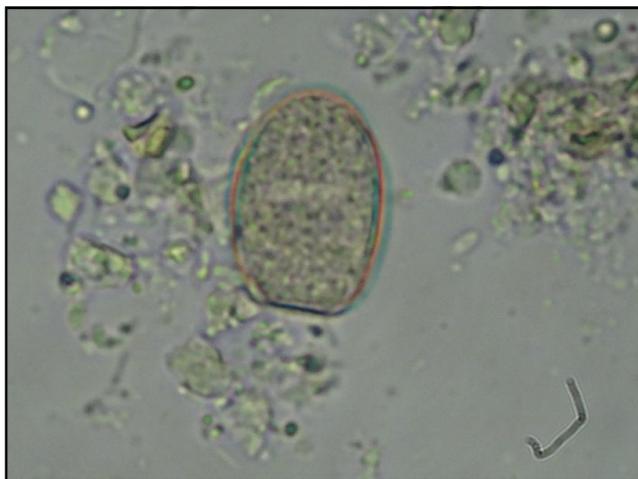


Fig. 5: Non sporulated *Eimeria cylindrica* oocysts by floatation with Sheather’s solution (X40).



Fig. 6: Sporulated *Eimeria cylindrica* oocysts by floatation with Sheather’s solution (X40).

Eimeria zuernii

Non sporulated oocysts:- Spherical to sub spherical shape, smooth wall colorless, polar cap absent, micropyle absent, medium size (15x13)µm.

-Sporulated oocysts:- appeared spherical to Ellipsoidal shape with 4 sporocysts each one contain 2 sporozites Fig. 8.

Discussion

Total rate of infection with *Eimeria* spp in buffaloes by traditional methods (Direct wet smear and Flotation sheather’s solution) in Al-Qadissiyyha province of Iraq was 53.8%. that result was different from those reported in Buffalo calves of Haryana (Rana *et al.*, 2011), who recorded low infection rate with *Eimeria* spp. (16.10%). Yadav and Sharma, (1986) suggested that inadequate feeding of colostrums, exposure to contaminated



Fig. 7: Non sporulated *Eimeria zurenii* oocysts by floatation with Sheather’s solution (X40).



Fig. 8: Sporulated *Eimeria zurenii* oocysts by floatation (X40).

environment, under feeding and poor sanitation are some predisposing factors for higher occurrence of coccidiosis in calves. High and low levels of prevalence have been confirmed in various studies concerning these protozoa in buffaloes like (49.6%) in Pakistan (Khan, 2012).

The rate of the present study is more than the rate of infection in which in Salah AL-dean province in 2015 by (Alani, 2015), which gave a rate of approximately 37%. The discrepancy between the rate of the present study and the rate of Al-any may be due to the substantial difference in sample size. Other possible causes for the low infection rate in Salah AL-dean province, might be due to environmental factors such as the greater humidity and relatively higher temperature in Al-Qadisiyha Province. These two factors may play a role in facilitating sporulation and Oocyst shedding. According to some studies, the variation in prevalence and distribution of coccidiosis may be attributed to the differences in management and hygienic conditions, temperature, agro ecology, climate, weather conditions, the immune state of the host, sample size, sampling period and breed susceptibility to coccidia in different areas (Altaf and Hidayatu, 2014). Dissemination of the *Eimeria* oocysts is feasible, especially in the highly productive crowded farms and readily introduces the highly infected rate (Hari *et al.*, 2010). The rate of infection in the present study varied according to age. The lowest rate of infection was recorded in buffalos more than three years of age and the rate was higher among young buffaloes, less than one year of age. Multiple studies proved the relation between highest rate of infection and the young age of buffaloes (Ribeiro, 2000; Nalbantoglu *et al.*, 2008). The young buffaloes are more susceptible to infection than older one due to immature development of the immune system of young animals in comparison with older animals. The young animal 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 to low dose infection is an important factor that make the animal more immune to a specific infection (Bahrami and Alborzi, 2013). The rate of infection in female buffaloes was significantly higher than that of male buffaloes, 58.3% versus 45%, in the present study. This finding is in accordance with (Priti *et al.*, 2008; Rehman *et al.*, 2011). This difference in rate of infection in females in comparison to males might be explained by the more stressful conditions experienced by female animals especially during pregnancy, delivery and breast feeding. The present study showed that the rate of infection was significantly high in March, April and Less in January. This implies that seasonal variation is an important factor that plays a role in the spread of *Eimeria* infection among buffaloes. This result is similar to the finding of many researches (Bilal *et al.*, 2009; Gupta *et al.*, 2011). The reasons for seasonal variation in rate of infection are thought to be due to variation in temperature, raining, moisture which may facilitate the maturation, shedding and sporulation of Oocysts. The relatively high temperature and dryness are important factors for low rate of infection in summer season. and may be due to warm and wet environmental conditions favoured by buffaloes which also proves conducive for the development of pre-parasitic free living stages of these parasites (Muraleedharan, 2005). This study showed no significant difference of prevalence *Eimeria* spp. among different regions at AL-Qadisiyha province. The differences could be attributed to the age, density, immunity of animals, management systems, methods of rearing and type of nutrition also the environmental conditions, the sampling method and sample size, as well as diagnostic techniques employed in different study localities, similar in these areas and this result is consistent with (Khan *et al.*, 2013; Al-Anai, 2014). The current study recorded four species of *Eimeria* in buffaloes according to the morphological characterization and measurements of oocysts by traditional methods (direct wet smear and floatation with Sheather's solution):- (*E. bovis*, *E. cylindrica*, *E. subsphrica* and *E. zurenii*). Oocyst of *E. bovis* appeared by microscope examination characterized by broad ellipsoidal to spherical shape and has smooth wall, pale-yellowish in color, medium size (28x20) μm , micropyle present, polar cap may be absent, *E. subspherica* oocyst appeared ellipsoidal shape very smooth wall colorless, polar cap absent, micropyle absent, medium size (12.5x10) μm , *E. cylendrica* oocyst appeared ellipsoidal shape smooth wall colorless, polar cap indistinct or absent, micropyle inconspicuous, medium size (20x12.5) μm and *E.zurenii* oocyst appeared spherical to sub spherical shape, smooth wall colorless, polar cap absent, micropyle absent, medium size (15x13) μm these results were compatible with the results of (Dubey *et al.*, 2008 Nain, 2014 and Alani 2015).

The young ruminants are more susceptible to infection than older one due 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 with older. The young ruminants 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 to low dose infection is an important factor that make the animal more immune to a specific infection (Yu *et al.*, 2011).

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