Prevalence of *Eimeria* parasite in cattle in Al-Najaf province and its relation to risk factors: age, gender and season

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Abstract

The study was designed to be an observational cross sectional study. The study extended from April 2015 through January 2016 and included 600 cattle with varying age (1 day to 8 years). It was conducted in Al.Najaf province and included four Districts: Najaf, Kufa, Al.Manathera, and Al.Meshkhab. Direct and flotation methods were used for the diagnosis of *Eimeria* infection. *Eimeria* infection was reported in 211 out of 600 (35.2 %). Infection rate was significantly highest in young cattle, less than three years of age (41%) (P<0.05). The infection was significantly more frequent in female than male cattle, 42.3% versus 22.6%, respectively (P<0.001). The highest rate of infection was reported during December (75%). Performance of Chi-Square test revealed no significant difference in rate of infection according to district (P= 0.632).

Key words: Eimeria, cattle, Al-Najaf, age, gender, season

معدل انتشار طفيلي الايميريا في الابقار في محافظة النجف وعلاقته بعوامل الخطورة : العمر والجنس والموسم

الملخص

تم تصميم الدراسة لتكون دراسة ملاحظة مقطعية. ولقد امتدت فترة العمل منذ شهر نيسان 2015 وحتى شهر كانون الثاني 2016 واجريت هذه الدراسة في محافظة النجف الاشرف وشملت الاقضية التالية: النجف و الكوفة والمناذرة والمشخاب. وشملت العينة 600 من الابقار تروحت اعمارها ما بين يوم وثمان سنوات. تم تشخيص الاصابة بطفيلي الايميريا بواسطة الفحص المجهري المباشر وطريقة التطويف. سجلت الاصابة بطفيلي الايميريا في 211 من النماذج الخاضعه للفحص وبنسبة (35,2%). كان معدل الاصابة عاليا وبصورة معنوية لدى الابقار التي يقل عمرها عن السنة بالمقارنة مع الابقار الاكبر سنا (P<0.05) وكذلك كان معدل الاصابة اعلى وبشكل معنوي في الاناث مقارنة بالذكور ، 42,3 % مقارنة ب 22,6% على التوالي (P<0.001).سجلت الاصابة الاعلى خلال شهر كانون الاول (75%). لم تكن هناك فروق معنوية في معدل الاصابة بين الاقضية المشمولة بالدراسة.(P=0.632)

Introduction

Coccidiosis is the result of infection by one of the species of *Eimeria* or *Isospora*, which infects a long list of mammals, especially the important domestic animals. The number of species of *Eimeria* in cattle is around 13 but they are mostly nonopathogenic. The mostly encountered species in disease causation is *E. zuernii* and to lesser extent is *E. bovis* (Norman and Levine, 1973: Vorste and Mapham, 2012).

It is well documented that this disease causes significant economic drawback through the great loss of domestic livestock especially in areas with limited or restricted environment and areas with great livestock densities. The economic drawback is related to the substantial mortality caused by infection added to the cost of treatment, on the other hand poor performance of the infected animal in growth and feeding is added to the economic loss. It is worth to mention that even the disease is subclinical, the animal will have poor growth rate due to reduced feed consumption and conversion and ultimately poor rate of growth (Lassen and Ostergaard, 2012).

The disease is characterized by variable patterns of severity depending on several recognized factors including host factors like the age of the animal and parasite factors like the dose of the parasite and its species and also factors related to obstacles

that interfere with proper management (Lalonde and Gajadhar, 2011).

The major observation is that the disease is common during the wet seasons of the year but it is of prime importance to consider the overcrowding as the main precipitating factor as it is well documented in calves brought together or domestic animals which are cared about in restricted small size environments and also in overcrowding around water sources (Daugschies and Najdrowski, 2005).

From clinical point of view the disease may be symptomatic in form of enteritis, nevertheless some cases are asymptomatic (Andrews, 2008). It is well documented that the disease is frequently encountered in goats, sheep and cattle but it is less frequent in horses (Cooke *et al*., 2013). *Eimeria* species are host specific beside they are characterized tissue tropism in such a way that they affect particular region of the intestine (Taylor, 2000). Thereby the aim of the present work was to study some epidemiological aspects concerning *Eimeria* infection in cattle in Al-Najaf province.

Materials and methods:

Study design

The study was designed to be an observational cross sectional study. The study extended from April 2015 through January 2016. The study included 600 cattle with varying age ranging from 1 day to 8 years. The study was conducted in Al.Najaf province and included four Districts: Najaf, Kufa, Al.Manathera, and Al.Meshkhab (figure 3-1).

Solution preparation
Formalin saline solution (10%)

This solution was prepared by added 100ml formalin (37%) into 900ml normal saline and mixed well. Then used in storage of feces and tissues specimen.

Sheather's Solution

This solution was prepared by dilution of 454 gm sugar in 355ml distilled water and mixed well in water bath at 100ºC. Then 6.7gm liquid phenol added.

The field study

Fecal sample collection

Ten gm feces samples were collected from rectum of each animal, and placed in sterile plastic containers and labeled for date, region, animal age, and gender according to special form for this study (Appendix I). Then each sample was transferred to the parasitology laboratory in the College of Veterinary Medicine/ Al-Qadissyia University and Al-Qassium Al-khdraa University. In the laboratory, direct wet smear was prepared for individual sample to detect infection under light microscopic examination. Two hundred eleven (211) samples were proved to carry infection by this method, and these were subjected to the subsequent steps of the study while the negative samples were properly discarded Then the samples that showed infection were examined by flotation method to prove the diagnosis with better morphology. The samples that proved to carry infection by direct smear and flotation methods were stored at -20 ºC for future genetic analysis.

Direct wet method: Adequate fecal sample was taken and then was put on glass slide. A cover slip was applied following addition of one drop of normal saline and was mixed by a wood stick. Examination was done with light microscopy at 10X and 40X Magnification powers (Coles, 1986, and Albakri, 2009).

Flotation method: The flotation method was used in some cases that cannot detection the *Eimeria* oocyst in direct smear. The flotation method was performed base on the use of Sheather's solution as following:

- A sample of 4.5gm feces was mixed with small amounts (10ml) of distilled water.
- The feces mixture was filtered by using sieve 40 anges to get rid of large particles.
- The filtrates were collected in sterile plastic tubes and placed in centrifuge at 1000 rpm for 3 minutes. Then the supernatant was discarded.
- Small amount of Sheather's solution was added into precipitate and mixed well by using wood sticks. After that it was placed in a centrifuge at 1000 rpm for 2 minutes.
- All plastic test tubes were placed on holder and stand vertical and drops of sheather's solution were added by pipetter until fill

the tubes. Then glass cover slide was placed on up end of tubes for 5 minutes.

- The glass covered slide was lifted carefully and placed under microscope at 10X, 40X, and 100X magnification power to look for the *Eimeria* oocysts (Ayez, 2006 and Al-Kabi, 2009)

Statistics analysis

Data were summarized, analyzed and presented using Statistical Package for Social Sciences (SPSS version 20) and Microsoft Office Excel 2010. Numeric variables were expressed as mean $\pm$SD (Standard deviation) while nominal variables were expressed as number and percentage. Chi-square test was used to study association between any two nominal variables while t-test was used to study difference in mean of numeric variables between any two groups. P-value was considered significant when it was equal or less than 0.05.

Results

Oocysts of *Eimeria* parasite were identified by direct wet smear and flotation methods using light microscope in a fresh fecal sample as shown in figure (1). The infection was reported in 211 out of 600 animals and hence the infection rate with *Eimeria* parasite was (35.2 %), as shown in figure (2).
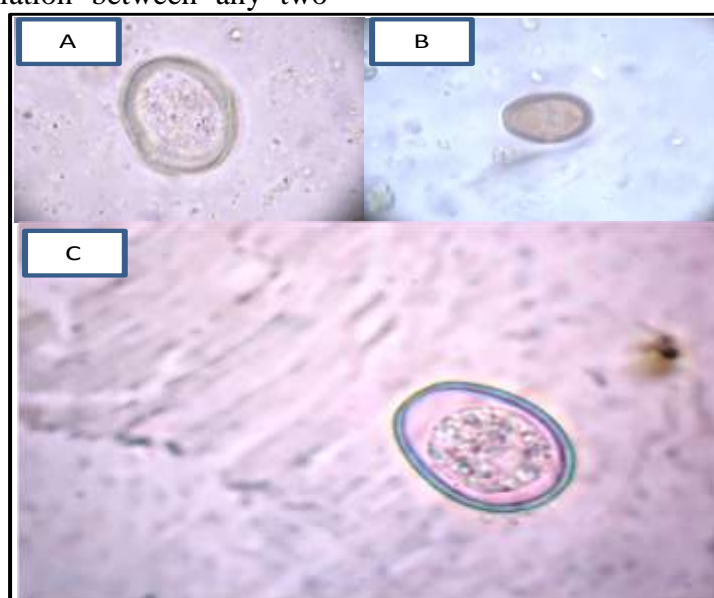

Figure (1): *Eimeria* oocyst detected by light microscopy using direct wet smear (A and B) and flotation (C) method 40X power.
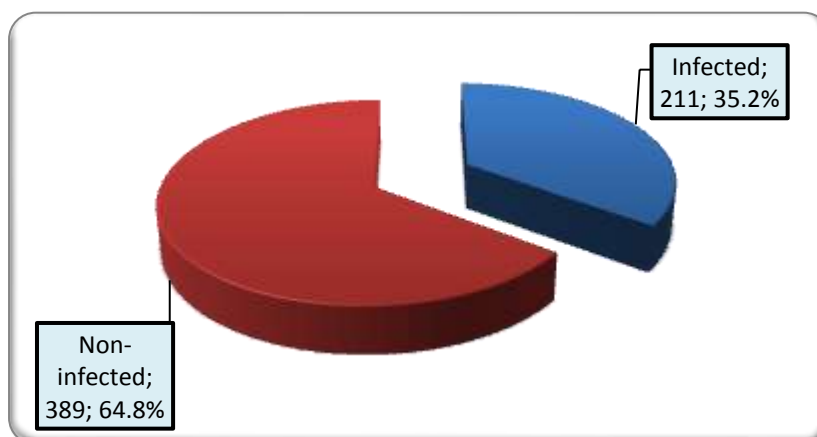

Figure (2): Pie chart showing the rate of *Eimeria* infection among cattle

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 (1). The first group, less than one year of age, involved 200 animal of which 76 animal proved to be infected by direct and flotation method and the rate of infection in this group of age was 38 %. The second group, one year up to < three years, involved 200 animals of which 82 proved to carry infection by direct and flotation method and the rate of infection was 41%. The third group, more than or equal to three years, was composed of 200 animal of which 53 were infected, and the rate of infection was 26.5% (figure 3).

The difference in the rate of infection in three age groups was statistically tested using Chi-Square test, which showed a significant difference (P=0.006), as demonstrated in table (1). In order to study the difference between any two age groups, with regard to infection rate, Chi-Square test was performed three times and the results are shown in table (2). First of all, there was no significant difference in rate of *Eimeria* infection between the first and the second group, 38% versus 41%, P=0.539, whereas there was a significant difference between the first and the third group and the second and the third group, 38% versus 26.5% and 41% versus 26.5%, respectively; P= 0.014 and 0.002, respectively.

Table (1): Rate of *Eimeria* infection in cattle according to age

Age interval	Total number	Infected animal	%
< 1 year	200	76	38.0
1 to <3 years	200	82	41.0
≥ 3 years	200	53	26.5
Total	600	211	35.2

P=0.006*

*Significant difference according to Chi-Square test

Table (2): Comparisons of *Eimeria* infection rate among individual age intervals

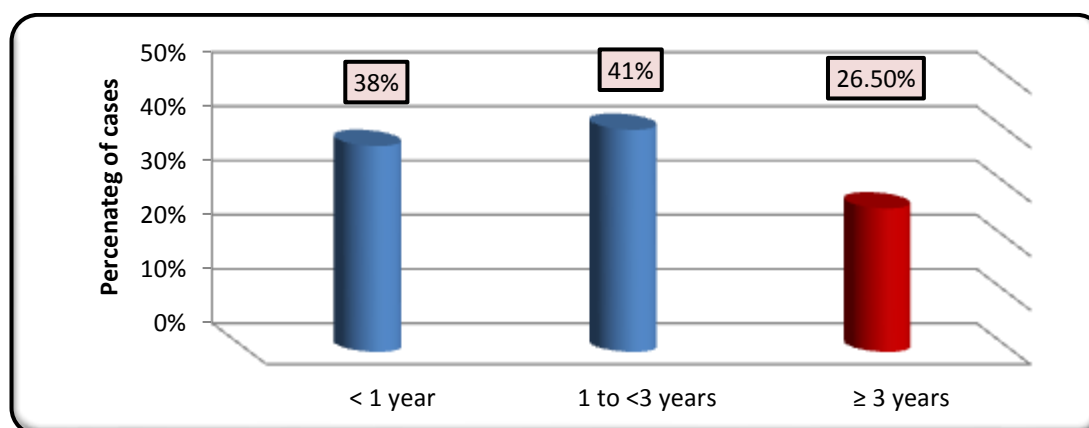| Age intervals	X^2	DF	P-value
<1 year versus 1 to <3 years	0.377	1	0.539
<1 year versus 1 to ≥3 years	6.053	1	0.014
1 to <3 years versus 1 to ≥3 years	9.403	1	0.002

Figure (3): Bar chart showing the rate of *Eimeria* infection according to age

The rate of infection according to sex was shown in table (3) and figure (4). Total number of female animals was 383 of which

162 were infected as proven by direct and flotation methods, whereas the total number of male animals was 217 of which 49 animals

had infection as it was proven by direct and flotation method. Accordingly the rate of infection in female animals was significantly higher than that in male animals, 42.3% versus 22.6%; P<0.001.

Table (3): Rate of *Eimeria* infection in cattle according to gender

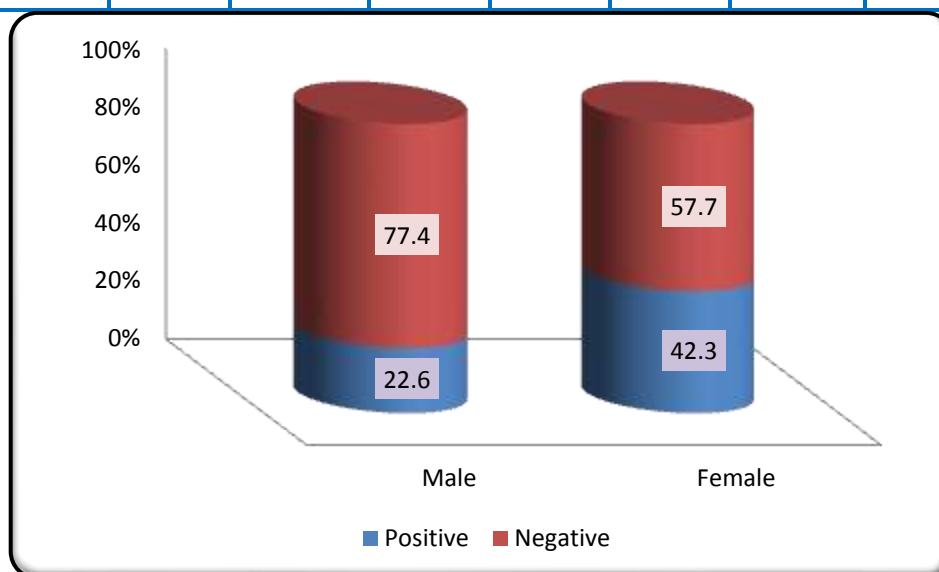|	Infection						X^2	DF	P-value
	Positive		Negative		Total				
Sex	N	%	N	%	N	%			
Male	49	22.6	168	77.4	217	100.0			
Female	162	42.3	221	57.7	383	100.0	23.619	1	<0.001
Total	211	35.2	389	64.8	600	100.0			

Figure (4): Rate of *Eimeria* infection in cattle according to sex

In order to study the variation in rate of infection according to sex in different age group, Chi-square test was performed between male and female animals in each age group, as shown in table (4). In the first age group, less than one year, there was no significant difference in rate of Eimeria infection between male and female animals, 38.8 % versus 37.6%; P=0.868. In the second age group, one year to less than three years, rate of infection in female animals was significantly higher than the rate of infection in male animal, 50.4% versus 23.9 %; P<0.001. In the third group, animals with age more than or equal to three years of age, the rate of infection in female animals was significantly higher than the rate of infection in male animals, 38.8% versus 7.6%; P<0.001, as shown in table (4).

Table (4): Rate of *Eimeria* infection according to sex by age groups

		Infection						X^2	DF	P-value
		Positive		Negative		Total				
Age group	Sex	N	%	N	%	N	%			
<1 year	Male	26	38.8	41	61.2	67	100.0	0.028	1	0.868
	Female	50	37.6	83	62.4	133	100.0			
1 to <3 years	Male	17	23.9	54	76.1	71	100.0	13.238	1	<0.001
	Female	65	50.4	64	49.6	129	100.0			
≥ 3 years	Male	6	7.6	73	92.4	79	100.0	23.96	1	<0.001
	Female	47	38.8	74	61.2	121	100.0			

The rate of infection according to month of the year is shown in table (5) and figure (5). July showed the least rate of infection which was 16.7%. The rates of infection in May, June, August, September and October were higher than that of July but the differences were statistically not significant, and the rates were 31.7%, 28.3%, 23.3%, 25% and 31.7% respectively, whereas the rates of infection during April, November, December and January were significantly higher than that of July and they were 35%, 40%, 75% and 45% respectively. The highest rate of infection was reported during December (75%).

Table (5): Rate of *Eimeria* infection according to month

Month	Total number	Number of infected animal	%	P-value*
April	60	21	35.0	0.035†
May	60	19	31.7	0.084
June	60	17	28.3	0.184
July	60	10	16.7	----
August	60	14	23.3	0.361
September	60	15	25.0	0.261
October	60	19	31.7	0.084
November	60	24	40.0	0.008†
December	60	45	75.0	<0.001†
January	60	27	45.0	0.001†
10 months	600	211	35.2	

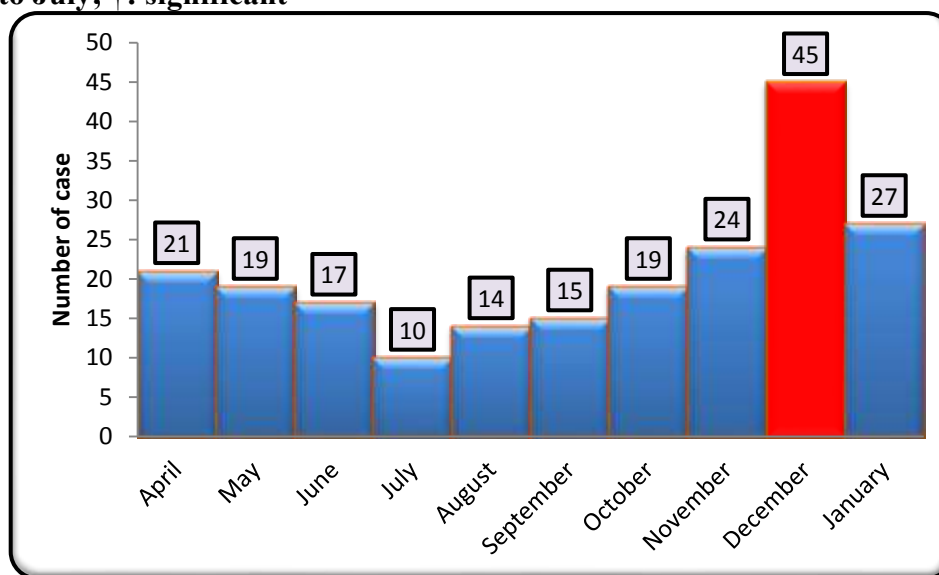*** Compare to July; †: significant**

Figure (5): Number of infected cases by month

The rate of infection in each district is shown in table (6). Total Cases collected from Najaf district were 92 of which 28 (30.4%) were infected. Total Cases collected from Kufa district were 246 of which 91 (37 %) were infected. Total Cases collected from Al-Manathera district were 155 of which 57(36.8%) were infected. Total Cases collected from Al-Meshkhab district were 107 of which 35 (32.7%) were infected. Performance of Chi-Square test revealed no significant difference in rate of infection according to district (P= 0.632).

Table (6): Rate of *Eimeria* infection according to district

District	Infected		Not infected		Total	
	N	%	N	%	N	%
Najaf	28	30.4	64	69.6	92	100.0
Kufa	91	37.0	155	63.0	246	100.0
Al-Manathera	57	36.8	98	63.2	155	100.0
Al-Meshkhab	35	32.7	72	67.3	107	100.0
Total	211	35.2	389	64.8	600	100.0

P=0.632

Discussion:

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. On the other hand the present study was designed to investigate the *Eimeria* parasite and hence the direct and flotation methods are sufficient, sensitive and effective in diagnosing *Eimeria* species.

By the use of direct and flotation methods, the rate of *Eimeria* infection was determined to be 35.2%. In the present study the total number of cattle was 600 and it was more than the least representative sample which was determined by the equation for calculation of statistical power. This equation gave a sufficient number of approximately 363, while the collected number was 600 which is far greater than the required sample size. Accordingly one can conclude that the estimated rate of infection by the present study is representative to the true rate of *Eimeria* infection in cattle all over the Country.

The rate of the present study is more than the rate which was estimated by Al-Bakry in 2009 who gave a rate of approximately 26%. The total number of cases which were included in the study done by Al-Bakry in 2009 was 140 which is less than that included in the present study. The discrepancy between the rate of the present study and the rate of Al-Bakry may be due to the substantial difference in sample size. Other possible causes for the low infection rate in Al-Mosel Province, as recorded by Al-Bakry, in comparison with result of the present study, might be due to environmental factors such as the greater humidity and relatively higher temperature in Al-Najaf Province. These two factors may play a role in facilitating sporulation and Oocyst shedding. Another important reason is the fact that Al-Bakry study extended from March through May, while the present study extended for ten months. From the result of the present study, it was obvious that March, April and May had a relatively low rate of incidence in comparison with December and January. Beside the managing routine may add to the difference in rate of infection.

Kshash *et al* in (2004) reported a rate of 21% in Al-Kadissiyah Province. This rate is less than the rate recorded by the current study, the difference is clearly due to the small sample size and the shorter duration of Kshash *et al.* study.

In Islamic Republic of Iran, the infection rate was estimated to be around 8.25% (Heidari *et al*, 2004) which is much less than the rate which was estimated by the present study. The sample size of the Iranian study was 400 and the study lasted for 3 years. So it is clearly that neither the sample size, nor the period of study can explain the big difference in the rate of *Eimeria* infection in cattle in Iran and Iraq. Other reasons should be taken into consideration, these include the enormous variation in environmental predisposing factors, like humidity and temperature, also the managing routines and methods and the hygiene and sanitation methods.

The prevalence in china was reported to be around 47.1% (Dong *et al*, 2012), which is clearly higher than that which was reported by the present study. The prevalence in Kenya was estimated to range from 30.9% to 67.4% (Munyua and Ngotho, 1990 and Waruiru *et al*, 2000). In Tanzania, the prevalence rate was estimated to be around 56% and the (Chibunda *et al*., 1997). In South Africa the prevalence rate was estimated to be approximately 52% in several states (Matjila and Penzhorn, 2002). The prevalence was reported to be around 59% in Japan (Oda and Nishida, 1990). In England the overall prevalence is around 43% (Mitchell *et al*., 2012). It was estimated that the overall prevalence of the disease in Germany was estimated to be 95.4% (Bangoura *et al*., 2011), While in Pakistan the prevalence was calculated to be around 47.1% (Rehman *et al*., 2011).

The variability in the rate of infection by *Eimeria* in cattle in different countries and from the present study may be due multiple factors. On the top of the list comes the variation in environmental factors, such as humidity and temperature. Difference in sample size also plays a role. The adoption of different managing routine strategies and hygiene and sanitation methods also could be blamed.

The rate of infection in the present study varied according to age. The lowest rate of infection was recorded in cattle more than three years of age, and the rate was higher among young cattle, less than three years of age. Multiple studies proved the relation between highest rate of infection and the young age of cattle (Faber *et al*., 2002, Daugschies and Najdrowski, 2005, Kennedy, 2007, Bandra *et al*., 2007, Klockiewicz *et al*., 2007, Ocal *et al*., 2007, Yu *et al*, 2011, Dawid *et al*., 2012 and Alemayehu *et al*., 2013).

The young cattle 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 (Yu *et al*, 2011and Dawid *et al*, 2012).

The rate of infection in female cattle was significantly higher than that of male cattle, 42.3% versus 22.6%, in the present study. This finding is in accordance with (Klockiewicz *et al*, 2007, Yakhchali, and Golami, 2008, Yu *et al*, 2011, Al.Jubori, 2012 and Dawid *et al*, 2012).

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. Beside that there is more or less better care applied to male cattle in comparison with female cattle, since male cattle are usually raised in closed barns and the feeding is more protein rich as these male animals are regarded important source of meat industry (Radostits *et al*., 2000, Yu *et al*, 2011 and Dawid *et al*., 2012).

The present disagrees with the result of (Warurin *et al*., 2000) who concluded that sex has nothing to do with the rate of infection, and also disagrees with (Craig *et al*., 2006) who stated that males are more affected than females.

The present study showed that the rate of infection was significantly high in November, December and January and Less in July. This implies that seasonal variation is an important factor that plays a role in the spread of *Eimeria* infection among cattle. This result is similar to the finding of many researches (Rodríguez *et al*., 1996, Warurin *et al*, 2000, Radostits *et al*., 2000, Daugschies and Najdrowski, 2005, Klockiewicz *et al*, 2007 and Al-Kabi, 2009). 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.

The present study disagrees with Alani *et al*. in (1989) who stated that the rate of *Eimeria* infection among cattle is not affected by seasons.

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