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Detection of *eimeria* species in domestic goat with both microscopic and molecular methods in Al-Diwaniyah Province

Wafaa Hussein Abdel Khader

Noor Idan Jarad
Email: noor.jarad@qu.edu.iq

***Abstract*---**The aim of this study which conducted in Al-Diwaniyah province during the period from October (2021) to April (2022) was to determine the prevalence of *Eimeria* infection in goat and study the effect of some epidemiological factor such as sex ,age and months on the infection rates addition to that and molecular identification of *Eimeria*. Two hundred and ten fecal sample were collected, from goat in four different regions in Al-Diwaniyah province included (Al-Shamiya, Al-Sunyih, Ghamas and Al-Mohanawih) at one visit per a week for each regions. The results of microscopic examination showed that 169(80.48%) of goat were infected. According to morphological study, eight *Eimeria* species were recorded which include *E. arloingi*, *E. alijevi*, *E. ninakohlyakimovae*, *E. christenseni*, *E. jolchijevi*, *E. hirci*, *E. caprovina* and *E. caprina*. The results showed that, *Eimeria arloingi* were recorded at highest rate (22.86%), while *E. caprina* was observed at lowest rate (3.33%). Significant (P<0.01) differences was found between prevalence of infections according to *Eimeria* species. Out of 120 fecal sample were collected from goat female and 90 fecal samples were collected from male which examined as *Eimeria* species, the results showed that, 99(82.5%) females and 70(77.78%) males were confirmed to be infected. No significant difference (p< 0.05) in the overall prevalence between males and females goat. Regarding to effect of age on the infection rate, the higher infection rate was recorded in animal aged less than 6 months (94.44%) and the age between (6-12) months in rate (80%), while the lower infection rate (50%) was recorded in animal aged more than 12 months. Statistical analysis of the data showed significant variation (P<0.01) on the overall prevalence of *Eimeria* species between age groups. The results showed that, the highest infection rate was observed in January and February in rate (96.67%), (93.33%) respectively, while lowest infection rate was observed in October and April in rate (63.33%), (66.67%) respectively with significant (P<0.01) differences. The current study recorded that the goat with the single *Eimeria* infection (57.62%) were

higher than those with the double infection (21.43%), triple (0.95%) and quadruple (0.48%) infection. Genomic DNA was extracted from 100 goat´s fecal samples and18S rRNA gene of *Eimeria* was amplified by polymerase chain reaction. PCR technique showed that, out of 100 goat´s fecal samples 87(87%) were positive for 18S rRNA gene of *Eimeria*.

***Keywords*---**PCR, eimeria, 18S rRNA.

Introduction

Coccidiosis is a parasitic disease caused by *Eimeria* species is a worldwide economically important parasitic disease with high prevalence in goats (Abo-Shehada and Abo-Farieham 2003; Rocha *et al.*, 2012). This disease leads to economic losses due to high mortality and morbidity, low growth performance, reduce productivity and treatment costs (Singh *et al.*, 2016). Taxonomy genus of *Eimeria* could be classify according to the morphological feature of speculated oocysts and host identify from which the oocysts have been recovered (Ogedengbe *et al.*, 2015).

Among the 17 species of *Eimeria* described in goats, *E. christenseni, E. arloingi, E. caprina, and E. ninakohlyakimovae* are considered to be highly pathogenic (Ruiz *et al.*, 2006). Goat *Eimeria* species have different reproductive rate and pathogenicity according to the site of infection, which result in different host pathophysiological responses and histopathological lesions (Hoste, 2001; Dai *et al.*, 2006).

The oocysts are passed in the faeces of infected hosts, goats are infected through the ingestion of speculated oocytes. In the small intestinal, speculated oocysts release sporozites and invade the intestinal epithelial cells, resulting in loss of electrolytes and nutrients malabsorption (Jubb *et al.*, 2007; McGavin and Zachary, 2011 and Temizel *et al.*, 2011).

The disease is more serious in 4–6 months old kids and also when animals of any age are kept in overcrowded houses and under stressor factors such as weaning, dietary changes; transportation, and cold or heat weather (Kaya, 2004; Gül, 2007). Temperature, moisture and oxygen tension are the main factors which determine the survival and development of coccidian oocysts to the infective stage (Ruiz *et al.*, 2012).

The optimum temperature for the sporulation of most *Eimeria* species oocysts of sheep and goats is 28-31°C temperatures below 40 and above 40°C are considered to be lethal (Kheysin, 2013). The most common clinical signs of infection with Eimeriosis are vary through reducing weight, anorexia, bloody diarrhea or not, and anemia (Wang *et al.*, 2010; Odden *et al.*, 2017).

Different diagnostic methods are available for specific identification of *Eimeria*, traditional methods are based mainly on oocysts morphological characteristics under microscopic examination, clinical signs and histopathology (Carvalho *et al.*, 2011). However, due to the presence of interspecies variation, the morphological

method is not fully reliable since natural infections by *Eimeria* are generally mixed with more than one species and several species have confusing features (Khodakaram-Tafti *et al.*, 2013). Molecular techniques have been reported as useful for species identification or classification of this genus of each *Eimeria* species (Yang *et al.*, 2014).

The 18S rRNA and its genes has been used extensively as a molecular marker in phylogenetic analysis (Ogedengbe *et al.,* 2011*).* For the purpose of the identification of species belonging to *Eimeria* parasite that effect the goat variations of this parasite in Al-Diwaniyah province this study was designed.

Materials and Methods

Fecal Samples Collection

Fecal samples (10-15) grams were collected from 210 goat at different ages, and of both sexes (males and females) during the period from the first of October (2021) to end of April (2022) from four different regions in Al-Diwaniyah province included four districts (Al-Shamiya, Al-Sunyih, Ghamas and Al-Mohanawih) at one visit per a week for each regions.

Fecal samples were collected directly from the rectum, in a 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 in refrigerated bags to a Parasitology laboratory in the College of Veterinary Medicine-University of Al-Qadisiyah.

Microscopic examination

Flotation technique is most commonly used in veterinary medicine for examination of feces; it is based on differences in specific gravity of parasite eggs (Dryden *et al.*, 2005). Floatation solutions include zinc sulfate, NaCl and Sheather's (Eckert *et al.*, 1995). Flotation method produce clear material than sedimentation for lighter egg amount, it is easy and inexpensive to perform (Christie *et al.*, 2011).

Molecular detection of the *Eimeria* oocyst

The primers were provided as lyophilized form and were dissolved in a high pure water to give a final concentration 100 Pico mole/µl as primer stocks. These were kept at – 20^{o}C until further use in a concentration (0.5 Pico mole/ 20 µl in total PCR reaction). These primers were supplied from Macrogen /Korea (Albanse *et al.*, 2019).

Preparation of master mix was done by (AccuPower® PCR PreMix). Composition of PCR premix tube is formed from (one U of Taq polymerase enzyme 1U, 250µM of dNTPs, 10mM of Tris-HCl ,30mM of 1.5mM of KCl, stabilizer, MgCl2 and dye). Preparation of master mix is done according to companys directions in total volume 20µl after adding extracted DNA 2µl, master mix 10µl, forward primer and

reverse primer at 1µl respectively .Completing the remaining size by deionizer water into 6µl then exposed for mixing by vortex (Bioneer company. Korea). The final reaction was done by using a thermocycler device(Mygenecompany. Made in Korea) depending on company instructions as following :

1. The first stage (denaturing stage) was done at 94°C for a 5 minute
2. The second stage consists of thirty-five cyclesat 94°C for 35 seconds .
3. The third stage (annealing stage) was done at 60°C for 30 seconds .
4. The fourth stage was done at 72°C for 45 seconds
5. The fifth stage (final extension stage) was done for 5minute at 72°C .

The electrophoresis was carried out using agarose gel 1% ;ethidium bromide dye was used for staining and watching under UV light device(table 1) .

Table 1: Primers of 18S rRNA gene of *Eimeria* species

Prime name	Sequence (5'-3')	Product Size
Eimeria 18F	CGCGCAAATTACCCAATGAA	450bp
Eimeria 18R	ATGCCCCCAACTGTCCCTAT	

Results

Prevalence according microscopic examination

Out of 210 faecal samples 169(80.48%) were found positive for *Eimeria* oocyst in microscopic examinationin (table 2).

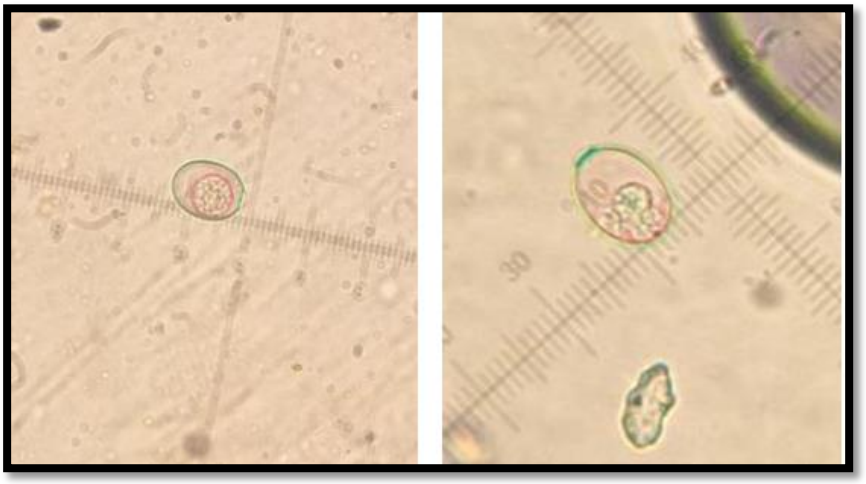
Table 2: The Infection with *Eimeria* Spp

Tested Samples Number	Positive Sample Number	Percentage
210	169	80.48%

During the microscopic examination of fecal sample, eight *Eimeria* species were recorded.

1. *E. arloingi*

Oocyst are ellipsoidal shape, yellowish brown in color and has a thick wall, with a micropyle cap and polar cap, and measure 24-36 x 16.2- 26 microns and sporulation time 2 day as shown in Figure (1).

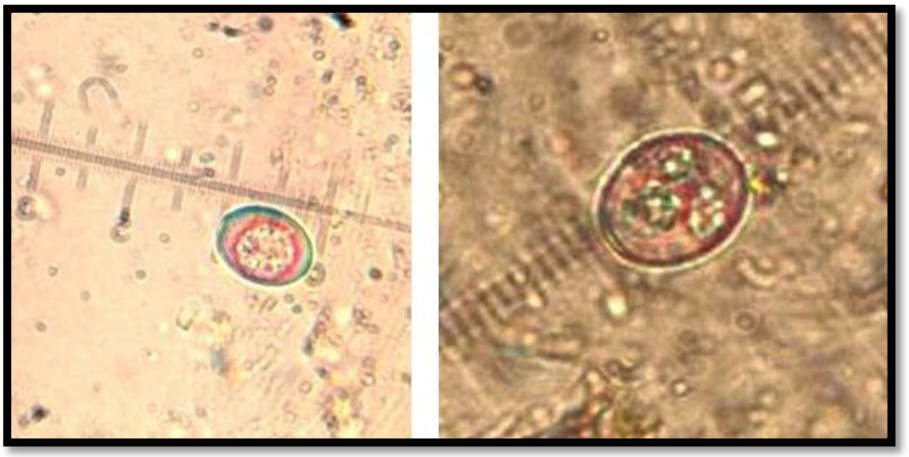


(A) (B)

Figure (1) *E. arloingi* (A) unsporulated (B) sporulated oocyst (40x)

2. *E. ninakohlyakimovae*

Oocyst appeared almost sub spherical to ellipsoid shape, greenish to brown in color and has a smooth wall, polar cap present and micropyle cap absent, they measured 20-27.3 x 14-21 microns, and sporulation time 4 day as shown in Figure (2).



(A) (B)

Figure (2) *E. ninakohlyakimovae* (A) unsporulated (B) sporulated oocyst (40x)

3. *E. christenseni*

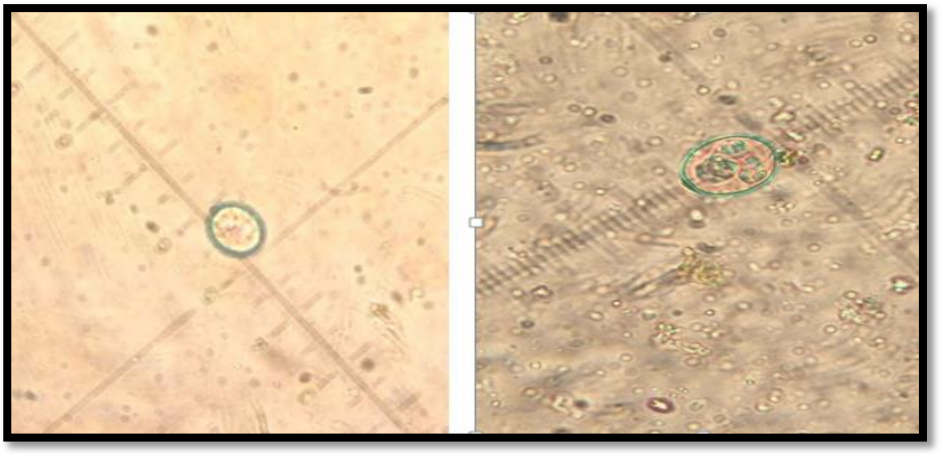
Oocyst of pear shape, yellowish brown in color, and has a thick wall with micropyle cap and polar cap, measuring 35-43.8 x 24-28.5 microns and sporulation time 4 day as shown in Figure (3).

(A) (B)

Figure (3) *E. christenseni* (A) unsporulated (B) sporulated oocyst (40x)

4. *E. hirci*

Roundish oval, greenish color, micropyle cap present, polar cap present but not clear, size 18-24 x 16-20 microns, sporulation time 3 day as shown in Figure (4).

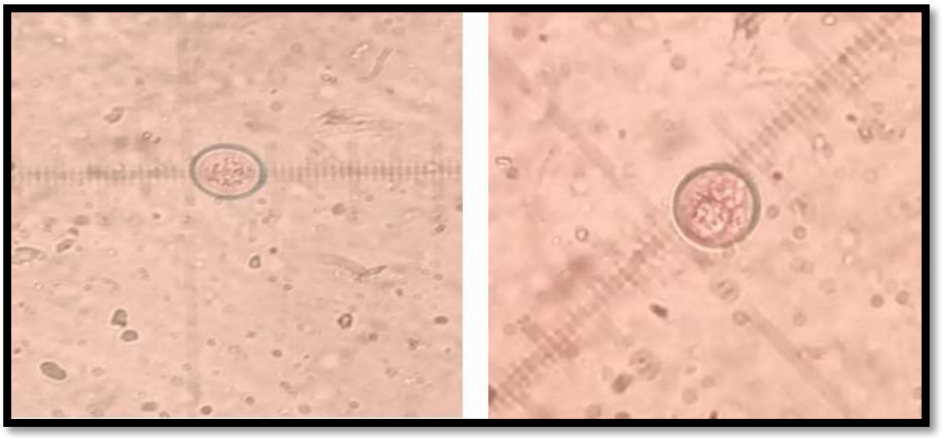


(A) (B)

Figure (4) *E. hirci* (A) unsporulated (B) sporulated oocyst (40x)

5 .*E. alijevi*

Oocyst sub spherical shape, two layer yellowish brown in color, and has a smooth wall micropyle cap and polarcap absent, measuring 16-23.7x 14-21 microns and sporulation time 2 day as shown in Figure (5).

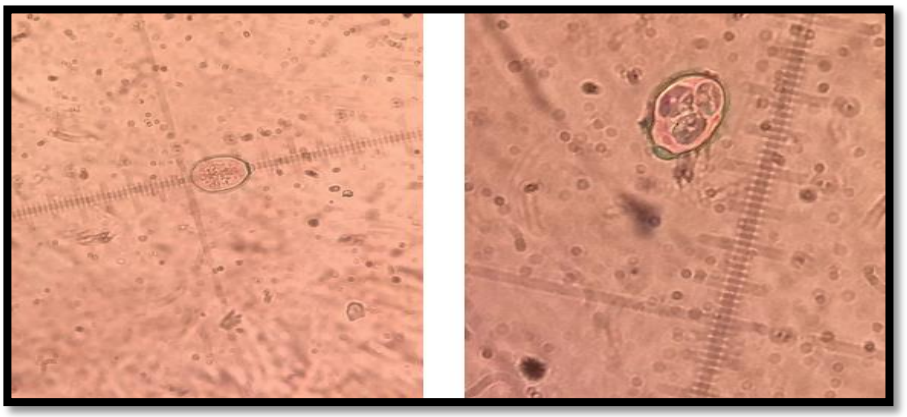


(A) (B)

Figure (5) *E. alijevi* (A) unsporulated (B) sporulated oocyst (40x)

6 .*E. jolchijevi*

Ovoidal to ellipsoid shape, brownish green in color, micropyle cap and polar cap present, size 25-34 x 19.5-26 microns, sporulation time 3 day as shown in Figure (6).

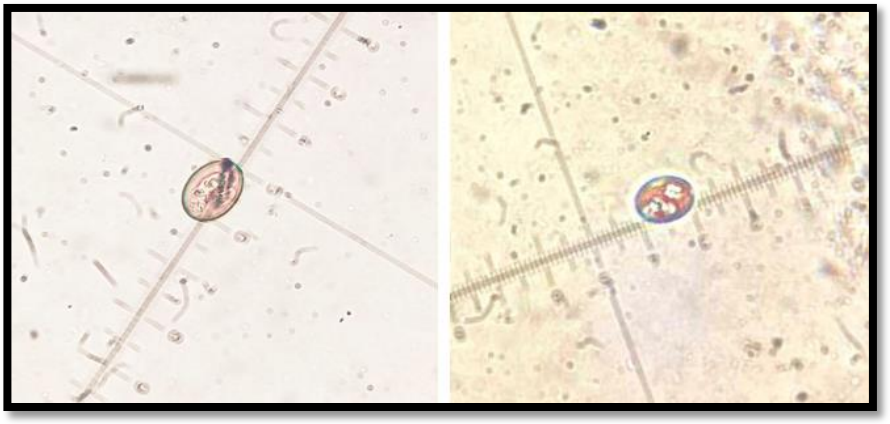


(A) (B)

Figure (6) *E. jolchijevi* (A) unsporulated (B) sporulated oocyst (40x)

7 .*E. caprovina*

Broadly ellipsoid shape, light pink in color, polar cap present and micropyle cap absent, measuring 27.36 x 20-27 microns and sporulation time 3 day as shown in Figure (7).

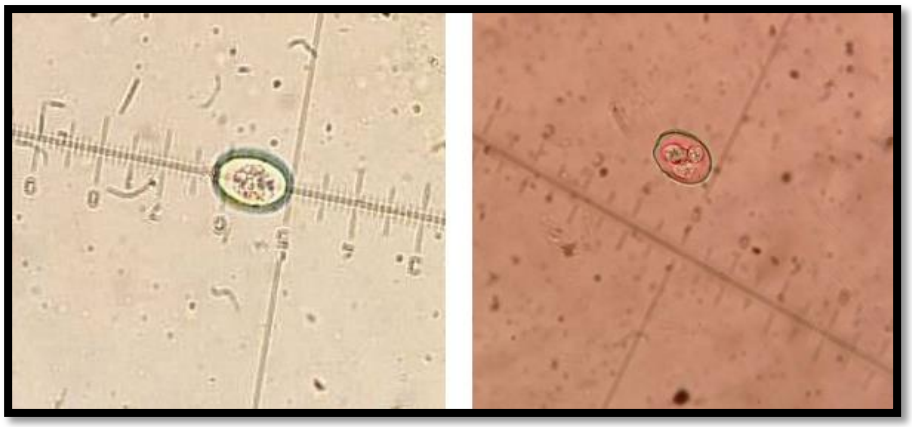


(A) (B)

Figure (7) *E. caprovina* (A) unsporulated (B) sporulated oocyst (40x)

8 .*E. caprina*

Oocyst ellipsoid shape, brownish yellow in color polar cap present and micropyle cap absent**,** measuring 28-39.5 x 20-25.5 microns and sporulation time 2 day as shown in Figure (8).



(A) (B)

Figure (8) *E. caprina* (A) unsporulated (B) sporulated oocyst (40x)

Infection rate of *Eimeria* species according to age

The higher infection rate was recorded in animal age less than 6 months (94.44%) and in the age range between (6-12) months in rate (80%), while the lower infection rate (50%) was recorded in animal age more than 12 months(table 3).

Table 3: Number of *Eimeria* infected goat depending on the animal age

Age	No. of infected goat	Total No. of Suspected infection	**(%)**
<6 month	90	85	94.44%
6-12 month	80	64	80%
> 12 month	40	20	50%
Total	210	169	80.48%

Infection rate of *Eimeria* species according to sex

Out of 120 fecal sample were collected from female goat and 90 fecal samples were collected from male goat which examined as *Eimeria* species, the results showed that, 99(82.5%) females goat and 70(77.78%) males goat(table 4).

Table 4: Number of *Eimeria* infected goat depending on the animal sex

Sex	Total No. of Suspected infection	No. of infected goat	(%)
Females	120	99	82.5%
Males	90	70	77.78%
Total	210	169	80.48%

Infection rates depending on PCR

Out of one hundred faecal samples 87% were found positive by PCR technique (Table 5; fig. 9).

Table 5: Total prevalence of *Eimeria* spp by PCR

The exanimated samples number	The positive samples number	%
100	87	87%

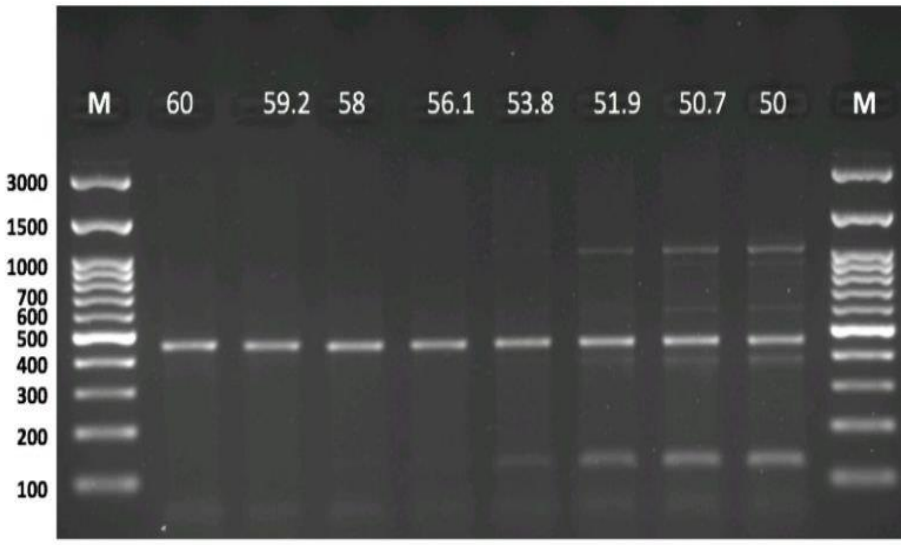


Fig.9: Gel electrophoresis image (1.5 % agarose) shows the amplicons of Emieria sp by gradient protocol for PCR optimisation. As can be seen, the optimal annealing temperature depicted as 60 C. M is molecular marker from AddBio, Korea.

Discussion

In the present study, investigation about *Eimeria* species in 210 fecal samples revealed high infection rate (80.48%). The high prevalence of *Eimeria* species of goats in Al-Diwaniyah province could be attributed to lower immunity of hosts as a result of malnutrition, grazing of young and adult animals together in poorly drained land provide an ideal condition for the transmission of the oocyst of endoparasites to build up clinical infestation of the host (Asif *et al.*, 2008 Gadahi *et al.*,2009).

The prevalence of *Eimeria* species found in this study was close to that reported in a previous study conducted in Baghdad, with an overall prevalence of 85 % from 180 goat fecal samples (Hasson,2022). Also in the Ethiopia, who recorded the prevalence of *Eimeria* species were 85.03 % from 384 goat fecal samples (Etsay *et al.*,2020).

The prevalence percentage of *Eimeria* species of goat in this study was less than that reported by Al- Bakray and Daoud (2005) in Mosul province, who recorded the prevalence of *Eimeria* species were 95.08% from 1140 goat. Study conducted in the in Southeastern, with an overall prevalence of 89.91 % from 208 goat fecal samples (Kheirandish *et al.*,2014). The infection rate of the present study was also higher than that reported in Iran 73.3% by Razavi and Hassanvand (2006), in Tikrit city 69.5% (Hasan and Mahmood, 2021).

The variation in the prevalence of *Eimeria* species infections between regions or countries could be explained by the differences in climatic factors required for the biology of the parasites and variations in the detection techniques (Bawm *et al.*,2020).

According to *Eimeria* species, the present study revealed that there are 8 different species of *Eimeria* infect goat, that which *E. arloingi*, *E. alijevi*, *E. ninakohlyakimovae* , *E. christenseni* , *E. jolchijevi*, *E. hirci*, *E. caprovina*, and *E. caprina*. So this result indicates that the study area may be endemic with different *Eimeria* species. The most previous researches indicated that there are any species of *Eimeria* that infected goat. In Al-Diwaniyah province, Ayiz (2006) was recorded eight *Eimeria* species, *E. arloingi*, *E. kochari*, *E. ninakohlyakimovae* , *E. christenseni*, *E. jolchijevi*, *E. hirci*, *E. caprovina*, and *E.apsheronica* . Wang *et al.* (2010) recorded 13 *Eimeria* species, Cavalcante *et al.* (2012) were confirmed 8 species in Brazil.

The present results showed that, *Eimeria arloingi* were recorded at highest rate (22.86%), while *E. caprina* was observed at lowest rate (3.33%). Other species were *E. alijevi* (13.33%), *E. ninakohlyakimovae* (10.48%) , *E. christenseni* (10%), *E. jolchijevi* (9.05%) , *E. hirci* (7.14%), and *E. caprovina*(4.29%). A suggestion of many species in goat should be expected, and putting it in mind, because of the nature of the studied animal and the measures of the oocyst. Hasson (2022) were recorded six species of the *Eimeria* in goat in Baghdad city, *E. arloingi* (39.21%), *E. alijevi* (24.83%) , *E. ninakohlyakimovae* (17.64%), *E. christenseni* (13.07%), *E. jolchijevi* and *E.bovis* (55.22%).

In Iran, Kheirandish *et al.* (2014) was recoded nine *Eimeria* species were identified consisting *E. arloingi* in rate (68.26%), *E. christenseni* in rate (50.9%), *E. ninakohlyakimovae* in rate (41.8%), *E. caprina* in rate (31.7%), *E. alijevi* in rate (29.8%), *E. jolchijevi* in rate (26.92%), *E. apsheronica* in rate (22.59%), *E. hirci* in rate (11.05%), and *E. pallida* in rate (5.2%).

There are various reports on prevalence rate of *Eimeria* spp. In Turkey, Gül (2007) detected 10 *Eimeria* spp. in goats such as *E. arloingi* in rate (47.43%), *E. christenseni* in rate in rate (45.14%), *E. ninakohlyakimovae* in rate (36.00%), *E. alijevi* in rate (26.85%), *E. hirci* in rate (23.42%), *E. caprina* in rate (18.28%), *E. caprovina* in rate (16.57%), *E. pallida* in rate (13.14%), *E. jolchijevi* in rate (10.28 %) and *E. apsheronica* in rate (3.42%).

A high relative abundance of *E. arloingi*, *E. ninakohlyakimovae* and *E. hirci* as well as a lower presence of *E. caprina*, *E. caprovina*, *E. alijevi*, *E. aspheronica* and *E. christenseni* has been commonly observed in goats under different environment (Cavalcante *et al.,* 2012), and probably it may be related to differences in *Eimeria* species virulence. Interestingly, the most pathogenic species in goats *E. arloingi* and mainly *E. ninakohlyakimovae*, which cause severe clinical signs (Koudela and Boková,1998).

Regarding to sex, out of 120 fecal sample were collected from goat females and 90 fecal samples were collected from males which examined as *Eimeria* species, the results showed that, 99(82.5%) females goat and 70(77.78%) males goat were confirmed to be infected. No significant difference ($P<0.05$) in the overall prevalence between males and females goat. These results were agreement with results obtained by Al-Bayati *et al* (2016) in Iraq who recorded the rate of infection of *Eimeria* species in both sexes was (95.7%) in males and (93.2%) in females, other results of Mohamaden *et al.* (2018) showed that, there was no significant

difference between males and females in the prevalence of *Eimeria* infection in goats. Ibrahim (2012) in Saudi Arabia who recorded the rate of infection in both sexes which was (55.19%) in males and (51.63%) in females with no significant difference. The infection rate which recorded in male and female in our study were different than other studied may be due to exposure different environmental condition and different management. Higher prevalence of *Eimeria* in female goats is in consistent with the findings of Hassanen *et al.* (2020).The result of this study was disagreement with Alali *et al.* (2021) in Iraq, who found that, sex had an influence on the prevalence of infection with coccidiosis in adult female goats, which were significantly higher than adult male goats; which is consistent with the findings of Kheirandish *et al.* (2014) from goats in Iran who recorded the rate of *Eimeria* spp. infection in female goats was higher than those in males. Also, Rehman *et al.*(2011) recorded the prevalence rate was high in females compared to males. Mohamaden *et al.* (2018) were mentioned that female are exposed to physiological stress in relation to pregnancy, giving birth and lactation that make it more susceptible to *Eimeria* spp. infection than males.

According to age, the higher infection rate was recorded in animal aged less than 6 months (94.44%) and in the age range between (6-12) months was (80%), while the lower infection rate (50%) was recorded in animal aged more than 12 months. Statistical analysis of the data showed significant variation ($P<0.01$) on the overall prevalence of *Eimeria* species between age groups. The result was agree with Hasan and Mahmood (2021) in Iraq, (Tikrit) which recorded high infection rate of coccidiosis in the young goats more than adult. These infection rates may occur due to the number of animals, animal's immune status, poor sanitary conditions and overcrowding.

A study of Mohamaden *et al.* (2018) found that, prevalence of infection rate of *Eimeria* species was significantly higher in adult (82.2%) rather than young goats (40%) . Shaheed and Al-Azizz (2020), Karawan, (2016) in Iraq, which recorded no statistical analyses between age groups. In other study by Yusof and Isa (2016), the maximum prevalence of coccidiosis was reported in goats below 6 months age and the lowest rate in group between 6–12 months age. A study of Zvinorova *et al.* (2016) found highest infection of *Eimeria* species in goat over than 3 years old and the lowest in animal aged 1–3 years old.

Bawm *et al.* (2020) found that, among the examined samples, (71.4%) of 77 kids were found to be positive, which is a higher association with *Eimeria* infection than weaners and adults with positive rates of (55.4%) and (55.9%), respectively. *Eimeria* infection was lower in adults probably due to more developed immunity in adults compared to kids (Silva *et al.*, 2014). This has been attributed to the high chance of exposure to the source of *Eimeria* spp. infection with increase in age and development of higher resistance or acquired immunity to coccidian in adult goats compared with those of kids.

According to study months, in average of 30 fecal samples for each month of period study were included seven months from October (2021) to April (2022) , the highest infection rate was observed in January and February in rate (96.67%), (93.33%) respectively, while lowest infection rate was observed in October and April in rate (63.33%), (66.67%) respectively with significant ($P<0.01$) differences

was found between prevalence of infections. In another study, Kheirandish *et al.* (2014) observed higher during the months of January and February. The results conducted by Kaldhusdal *et al.* (2021) was recorded high infection rate in December (90%). Higher prevalence of coccidial infection was in wet months compared to winter months was reported by various studies (Balicka-Ramisz, 1999; Manya *et al.*, 2008 and Mai *et al.*, 2009) .Also agreement with Al-Saadoon and Al- Rubaie, (2018) in Iraq which recorded the higher infection rate during the cold wet season and lower during the hot dry season. Swarnkar and Singh (2020) were recorded the high infection rate in March. Mohamaden *et al.* (2018) were recorded about (74.2%), (38.2%), (43.2%), (75%) of sheep and (66%), (43%), (44%) and (65%) of goats were infected with *Eimeria* in autumn, winter, spring and summer, respectively. Moore *et al.* (2018) revealed that, the highest rate of infection was in winter (83.1%) followed by spring (80.8%). While the lowest rate was in summer (61.2%). The high infection rate of *Eimeria* during the cold wet season may be due to climatic conditions which were more suitable for sporulation and survival of coccidian oocysts.

Alcala-Canto *et al.*(2020) were mentioned that the summer and autumn are the most seasons where *Eimeria* infection is more likely to occur. It might be because summer where the climate there is very hot and that might be a stressful factor to the animals that leads to more shedding of the protozoa, while autumn shows an increased level of infection due to the humidity which is more favorable for sporulation of oocysts.

Regarding to type of infection, the result of the present study demonstrated that, the single *Eimeria* infection was more common (57.62%), than the double (21.43%), triple (0.95%) and quadruple (0.48%) infection. Significant ($p<0.01$) difference was observed .The result was similar to other researchers as Hasan and Mahmood (2021) in Iraq (Tikrit) and Ibrahim (2012) in Saudi Arabia, .Also ,the present results were agreement with results of Sufi *et al.* (2017), who found that, the occurrence of single-species of *Eimeria* infection was higher than mixed-species infection. The reason might be the rearing system in the study area. Shaheed and Al-Azizz (2020) showed that, single infection of *Eimeria* was (0%) of infected goat, double infection was (57.14%), while mixed infection rate was (42.85%).

Satish *et al.* (2019) found that, (28.57%) as single infection of *Eimeria*. The mixed infection has been observed in study of Al-Bayati *et al.* (2016) which were dealing with the same subject. The higher rate of mixed infections in goats was consistent with previous research reports as Kahan and Greiner(2013) and Mohamaden *et al.*(2018) and could be attributed to the intensive management system which increased possibility of infection with various types of *Eimeria* spp. (Khodakaram-Tafti and Hashemnia, 2017). The *Eimeria* species mixed infection occurrence might be attributed to free feeding style which was dependent on the geographical area of this work. Diagnosis of *Eimeria* spp. oocyst by floatation methods based on morphological features can be difficult to discriminate (Khodakaram-Tafti *et al.*,2013).

According PCR based on amplification of DNA that has been used for the diagnosis of *Eimeria* parasites in animals. A number of approaches have proved to

be both specific and highly sensitive for analyses either of parasites grown in vitro or present in tissue samples and clinical materials (Kawahara *et al.*, 2010). PCR based techniques, have been developed and used for accurate identification and diagnosis of *Eimeria* spp. because of their high sensitivity, specificity, rapidity and utility (Yang *et al.*, 2014). The total results of PCR technique showed that, out of 100 goat fecal samples 87(87%) were positive for (18S rRNA) gene. Study in Baghdad by Hasson (2022) was recorded 93 out of 100 (93%) of samples were positive for *Eimeria* spp. using the 18sr RNA gen. There is little Molecular studies on the infection rate of *Eimeria* species in goats in world and according to our information the first in Al-Dwiniyah province .In Myanmar, Bawm *et al.* (2020) was recorded 93 out of 100 (93%) of samples were positive for *Eimeria* spp. The difference in results of infection rate may be related to the study sample size which examined.

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