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

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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 (3.3%). 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 and 18S 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**—detection *Eimeria* species, domestic goat, microscopic, molecular methods.

**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 sporozoites 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°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 (Mygene company. Made in Korea) depending on company instructions as following:

1. The first stage (denaturing stage) was done at 94°C for 5 minutes.
2. The second stage consists of thirty-five cycles at 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 5 minutes 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>
<thead>
<tr>
<th>Prime name</th>
<th>Sequence (5’-3’)</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eimeria 18F</td>
<td>CGCGCAAATTACCCAATGAA</td>
<td>450bp</td>
</tr>
<tr>
<td>Eimeria 18R</td>
<td>ATGCCCCCAACTGTCCCTAT</td>
<td></td>
</tr>
</tbody>
</table>

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

Results

Prevalence according microscopic examination

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

<table>
<thead>
<tr>
<th>Tested Samples Number</th>
<th>Positive Sample Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>169</td>
<td>80.48%</td>
</tr>
</tbody>
</table>

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

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).
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).

E. christensenii

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).
**E. christenseni**

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).

**E. hirci**

Oocyst sub spherical shape, two layer yellowish brown in color, and has a smooth wall micropyle cap and polar cap absent, measuring 16-23.7x 14-21 microns and sporulation time 2 day as shown in Figure (5).
Figure (5) *E. alijevi* (A) unsporulated (B) sporulated oocyst (40x)

**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).

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

**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).
E. capriona

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).

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).

Table3: Number of Eimeria infected goat depending on the animal age

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of infected goat</th>
<th>Total No. of Suspected infection</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6 month</td>
<td>90</td>
<td>85</td>
<td>94.44%</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total No. of Suspected infection</th>
<th>No. of infected goat</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>120</td>
<td>99</td>
<td>82.5%</td>
</tr>
<tr>
<td>Males</td>
<td>90</td>
<td>70</td>
<td>77.78%</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>169</td>
<td>80.48%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>The examined samples number</th>
<th>The positive samples number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>87</td>
<td>87%</td>
</tr>
</tbody>
</table>

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 many 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 coccidial 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.

**References**


