Microscopic and molecular diagnosis of *Eimeria* spp. in sheep as a model of health investigation

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Abstract
Coccidiosis is a parasitic disease caused by the *Eimeria* spp. that affects sheep and ruminants and leads to important economic losses. Studying of incidence of coccidiosis will help us to decrease the coccidiosis infection in the sheep flocks, and resulting in novel control programs. The *Eimeria* prevalence in sheep is taken in the Diwaniyah city from March 2017 to February 2018. 200 sheep were used in this study (95 Lambs and 105 Adult) that tested clinically by taking fecal samples and examined by a routine microscopic examination and conventional PCR. Results revealed that 57.5% of sheep were infected by the *Eimeria* parasite. Adult female sheep were more infected 77.9% than adult male sheep 41.3%, but in Lambs the parasite was higher in males 62% than the male 44.4%, also the overall prevalence of *Eimeria* spp. according to age was higher in adult’s 60.9% than that in Lambs 53.6%. In conclusion, gender significantly affected the prevalence and severity of the infection. Age, possibly, might be a significant factor adding to the notable losses in sheep in the Diwaniyah city in Iraq.

Key words: Detection, *Eimeria* spp., sheep, Iraq


Introduction
Sheep are an essential part of the livestock in many countries of the world and play role in the lives of their citizens and food security, as the Arab country has about 118 million heads of sheep, and Iraq has about 10.5 million sheep [¹]. The economic importance emerges for sheep in being the main source of meat in many Arab countries, accounting for 48% of the total meat consumed in developing countries. Sheep are ranked third after cows and buffalo in terms of providing red meat [²]. Like other farm animals, sheep herds are affected by various types of diseases such as parasitic diseases, which adversely affect the health and productivity of animals and the most important of these diseases is infected by gastrointestinal tract parasites (GIT) [³]. The coccidiosis is affected by invertebrates and vertebrates and caused by intracellular protozoa. The man could also be struck by this disease [⁴, ⁵].

The coccidiosis in sheep is caused by *Eimeria* genus; most common of the animals have coccidia, but it exhibited no clinical signs [⁶]. That called subclinical coccidiosis, that causing great loss economics, wherever it results in losing weight, increased susceptibility to some diseases and feeding inefficiency. The coccidiosis causes great financial losses because of treatment costs, growth decreases and high mortality.

The observed morbidity usually between 10- 40 %, but mortality often is more than 10% [⁷, ⁸]. The *Eimeria* affect all ages, especially the lambs [⁹]. The rigor of clinical signs depends on the capability of the host and the infecting dose [¹⁰]. Emirates causes diarrhea, which occurs for one week or more and leads to death [⁷, ¹¹]. Small ruminants under four months of age may be killed by Coccidiosis [⁷]. In our current study, we
adopted the method of light microscopy as a routine method to diagnose the Eimeriais, which is a fast and low-cost method in addition to the use of conventional PCR method, which is of high accuracy in detection the different Emiria spp.

**Materials and methods:**

**Fecal samples**
A total of 50 fecal samples is collected from sheep. Some of these sheep were suffering from diarrhea and other asymptomatic in the slaughterhouse and other areas in the Diwaniyah provinces from October 2017 to February 2018 from age 1≤ 1 year to more than 1 year. These samples were collected in the sterile plastic containers and stored in the large containers containing ice bags, then transported to the parasitology laboratory in veterinary medicine, Al-Qadisiyah University to perform the examination.

**Floatation method**
Feces sample 2 gram was added to sugar solution 10 ml for make flotation methods in a cup then centrifuged for separation two layers, the liquid remaining in the gauze strainer was squeezed from the feces by tongue depressor, then centrifuged 1,000 rpm for 5 min, the tube was removed, put in a test-tube rack, and filled to the top with sugar flotation solution, a 22 x 22 mm cover was put on the tube, left for 10 minutes, removed and placed on a glass slide, the entire coverslip was then examined under a light microscope \[12,13\].

**Microscopic diagnosis**
The identification of the species was done with respect to the morphology of sporulatedoocysts under the light microscope. Morphological characteristics: size, shape, color and wall thickness of oocysts, size, and shape of sporocyst and sporozoite, presence and absence of stadia, granular pole, micropile and cap \[14, 15\].

**Sedimentation method**
Fecal samples were processed by the fecal centrifugal sedimentation method as described\[16\].

**Molecular detection of the Eimeriaocysts**
PCR reaction was done by use designed primers for diagnosis of *Eimeria sp*, based on (ITS-1) area. In this study, a specific pair of primers were designed based on \[17\] and provided by (Bioneer Company, Korea). The primers were used to amplify 348 bp of (ITS-1) region in Eimeria sp. ITS-1primer (F: 5´- GCA AAA GTC GTA ACA CGG TTT CCG -3´) and 18srRNA-R primer (5´- CTG CAA TTC ACA ATG CGT ATC GC-3). The preparation of the master mix was by using a kit called (AccuPower® PCR PreMix kit. Bioneer company, Made in Korea). The premix consists of the polymerase enzyme, KCl, dNTPsMgCl2, stablizer, ethidium bromide, and Tris-HCl, and the reaction is done depending on company direction wherever the total volume was 20 µl, it consists of extracted DNA and reverse and forward primers, after that completing premix tube with water then mixed by a vortex.

The reaction was done in thermocycler apparatus (MygeneBioneer Company) as the initial denaturing stage at 94 °C for thirty seconds, 35 cycles at 94°C for ten seconds, the annealing stage 55 °C for twenty seconds, the elongation stage 72°C for twenty seconds, the extension at 72°C for two minutes. The final products were tested by electrophoresis and dying by ethidium bromide, and detect under UV light Trans illuminator.

**Results**

**Morphological Detection of Oocysts**
Oocysts of *Eimeria sp*. were found in 57.5% (115/200) of the examined fecal samples from diarrheic sheep using a light microscope (Fig. 1), (table 1). The overall prevalence of Eimeria sp. according to age was higher in Adults 60.9% than that in Lambs 53.6% (table 2). The *Eimeria sp*. were more prevalent in male hosts 62% as compared to female hosts 44.4% in Lambs, but in Adult, the parasite was higher in female 77.9% than the male 41.3% (table 3).

| Table 1: The prevalence of *Eimeria* infection in the examined sheep. |

No. of sheep examined | Infected with *Eimeria* spp. | Free from parasite (microscopically)
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive No.</td>
<td>Prevalence %</td>
</tr>
<tr>
<td>200</td>
<td>115</td>
<td>57.5</td>
</tr>
</tbody>
</table>

**Table 2:** The prevalence of *Eimeria* infection in the examined sheep in relation to age.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Examined animals</th>
<th>Positive No.</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs</td>
<td>95</td>
<td>51</td>
<td>53.6</td>
</tr>
<tr>
<td>Adult</td>
<td>105</td>
<td>64</td>
<td>60.9</td>
</tr>
</tbody>
</table>

**Table 3:** The prevalence of *Eimeria* infection in the examined sheep in relation to gender.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>Lambs</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals</td>
<td>Positive No.</td>
<td>Prevalence %</td>
</tr>
<tr>
<td>Males</td>
<td>50</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Females</td>
<td>45</td>
<td>20</td>
<td>44.4</td>
</tr>
</tbody>
</table>

**Molecular Detection of Oocysts**

The conventional PCR assay was carried out to detect the species contained in them. The amplicons of size 348 bp of (ITS-1) region were showed a clear single specific band (Fig. 2).

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**Fig. 1.** The osocyst of *Eimeria*.

**Fig. 2.** PCR amplification utilization...
fetal samples using the ITS-1 PCR reaction DNA ladder are located on the left side of the gel, fragment sizes are represented in base pairs (bp) 1:6 samples.

**Discussion:**

Our study reveals a moderate rate of Eimeria infection in the Diwaniyah city, although the hardest environments in the city, the study conducted that the prevalence of Eimeria in sheep has affected all genders, age. However, determining the prevalence of coccidiosis has great benefit in determining the perfect control strategy and for decreasing the economic losses. In the present study, 200 fecal samples of sheep were examined 115 of them are infected by the Eimeria parasite with a total percentage reach to 57.5 %. Respecting of Eimeria spp. infection, our result corresponds with [18] which showed that the incidence was 57.70% in sheep in Geneffe village, Suez Governorate, Egypt While these results disagreed with the results of which recorded prevalence of coccidiosis 72% in sheep in Sulaimaniya Province [19,20,18].

Also, disconformity with the findings from [21] which reported prevalence rate 67.5% of coccidia in sheep in the province of AL-Muthanna but in Garmiyan Province, Kurdistan region recorded a low ratio of coccidiosis in sheep about 31.30% [22] all these reports recorded in Iraq, as well as in sheep of other countries. In Iran, the rate of Eimeria in sheep was 240 positive, the total rate of coccidiosis in sheep was 19.2% [23]. And in the Colombian Northeastern Mountain [24] which recorded prevalence of Eimeria spp. 30.9% in sheep. Environmental and seasonal conditions, management and husbandry systems and number of samples collected can be the most important factors behind the variance in results. In Iraq, sheep usually graze in open spaces and get drinking water from streams and streams of small rivers and are therefore susceptible to many intestinal parasites [25].

While in most countries of the world are fed herd sheep in farms built and controlled and this affects the transmission of infection and spread among members of one herd, taking into account the role of drinking water and feed in the spread of infection in the case of contamination with the contagious stages of the parasite [26-29]. Our results showed that age and sex have a great effect on infection rates with coccidiosis in ewe 77.9%, wherever it was higher than rams 41.3%. Research proved that female animals were more sensitive than males against infection. For example, in Spain: females infected at 49.26%, and males at 28.27% [22], in Iran, females infected at 54.34%, and males at 45.65% [30], while in India: females infected at 64.3%, and males at 27.5% [31]. Furthermore, in Turkey: females infected at 91.4%, and males at 86% [32,33], but in Lambs Infection in male higher as compared to female hosts. Detection of the disease morphologically, by flotation methods by using saturated fluids good for Eimeria diagnosis, but it required time and experience [32].

Our report aimed to diagnose the Eimeria infection by using PCR, wherever it considered modern molecular methods for amplification of DNA after extraction it [34, 35]. Our study demonstrates most cases were subclinical, results of PCR have very sensitive and highly effective after washing and sporulation of the assets which results in the removal of all inhibitors of PCR, including glycogen, enzymes, fats, minerals and polysaccharides which present in fecal samples [36]. All samples were positive by using PCR, but the status is reversed by using fecal samples due to DNA increases after inhibitors and washing after the sporulation. So, sporulation of Eimeria oocysts before genetic material extraction has a role in subclinical conditions lead to fewer numbers of oocysts. Eimeria lives and multiple on the host without marked pathogenicity. This balance between parasite and host is disorder, especially when the host exposure to the stress, such as weaning, illnesses, food changes, and sharp climatic changes, leads to the multiplication of the parasite more than other cases. Management of the farm should be dependent on hygienic measurements, which will reduce the economic losses which occur due to coccidiosis [37].

**Ethical approval**

This manuscript has not been previously published in any language and is not under consideration in the same or substantially similar form in any other peer-reviewed media. The author declares that this study is a single study and not split up into several parts. The authors would like to confirm that the results of this study were presented clearly, honestly, and without fabrication, falsification or inappropriate data.
manipulation (including image based manipulation). There is no data, text, or theories of others are presented as if they were the author’s own.

**Animal Rights Statement**
The experiments on animals were conducted in accordance with the local Ethical Committee laws and regulations as regards the care and use of the animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were adopted. All procedures performed in this study were in conformity with the ethical standards of the establishment or practice at which the subject fields were carried.

**Conflict of Interest:** The author declares that they have no conflicts of interest.

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**References:**