11

Eimeriosis in Small Ruminants in Basrah Province/Southern Iraq

AUTHORSDETAIL

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INTRODUCTION

Livestock is one of the most important sources of the economy for any country. So, it is necessary to ensure the good health of animals, their development and prosperity and to preserve it from wasting and death. This is only possible by the periodic examination to ensure that it is free from bacterial, viral and parasitic diseases and focus on giving vaccines on time. Parasitic infections among small ruminants play a significant role in animal death and productivity, and *Eimeria* is one of the parasitic protozoa with a wide spread epidemiology among all animals, including small ruminants. The rates of its spread among animals have increased recently, and the reason for this is the spread of random grazing and the dependence of shepherds on feed from contaminated sources. Another reason for the increased infection is the mixing of animals in the same barns and lack of ventilation leading to the massive spread of sporozoites and emergence of new species that did not exist previously. Therefore, it is necessary to give the the utmost importance to this subject, and to follow up on the frequency of *Eimeria* between this region, and to find solutions to eliminate the parasitic infection. The emergence of new types of *Eimeria* was noted when it was detected at the molecular level. Formerly, eimeriosis was thought to be caused by the obligatory intestine intracellular apicomplexan protozoan parasite *Eimeria spp.* (Yakhchali and Rezaei 2010). The disease rapidly spread throughout the world and afflicted many animals, costing both individual farmers and the ovine business very badly (Reeg et al. 2005). *Eimeria spp.* is a parasite that infect several domestic animals, with the site of infection being the gut and occasionally other organs, including the liver and kidney (Levine 1973). Taxonomically, *Eimeriaspp.* has been placed in the *Eimeriidae* family including more than 1,000 species and the genus *Eimeria* comprising the majority of species affecting domestic animals as well as birds. There is total 15 species known to infect the sheep, however, *Eimeria* (*E.*) *ovinoidalis* and *E. crandallis* are the two most dangerous species (Catchpole et al.2000). There are 17 species known to have been found in goats, although the pathogenic species *E. arloingi* and *E. ninakohlyakimovae* are particularly common (Cavalcante et al. 2012). In life cycle, Oocysts are excreted in the faeces of infected animals and require favourable environmental conditions, such as temperature > 15°C and relative humidity > 80%, to mature into Sporulated oocysts that are capable of infecting other animals in the same field (Daugschies and Najdrowski 2005). Additionally, the principal route of transmission of disease between animals is through the ingestion of contaminated food and water containing oocysts (Fitzgerald 1980).

Historical Preview

The first discovery of *Eimeria spp.* was documented in 1674 by Antonie Van LeevnHook, who examined parasitic cysts in gall bladder ofrabbits. Then, schizogonous stages was descript by Schneider in 1875.Later, avian *Eimeria* oocysts was described by Leuckartin 1879.Schaudinn documented the whole life cycle of the parasite in 1900; thereafter, *Eimeria* was regarded as a distinct species from Eimerian, and the term *Eimeria* was first recorded in 1902 by Stiles and Liihe. The first discovery of *Eimeria spp.* in goats was documented by Marotelin 1905, who give it the name *Coccidiaumarloingi* having the Micropyle. The pathogenic aspects were clearly described by Johnson in 1930 and Tyzzeret al. 1932 (Soulsby 1974).

*Eimeria spp.*in Sheep and Goats

Different species of *Eimeria* found and describe in sheep and goats around the world (Sweeny et al. 2011). In sheep, Fifteen species of *Eimeria* was described by Soulsby (1982), like: *E. ahsata*was described by Honess(1942), *E. ovina* by Levine and Ivens (1970), *E. ovinoidalis*by by Yakimoff (1933), *E. crandallis* by Honess(1942), *E. faurei* by Moussuand Marotel(1902),*E. gilruthi*by Martin (1909) and Chatton (1910), *E. gonzaelzi*by Reichenowand Carini (1937), *E. granulosa* by Christensen (1938), *E. hawkinsi* by Ray (1952), *E. intricata* by Spiegl (1925), *E. pallida* by Christensen (1938), *E. parva* by Kotlân et al. (1951), *E. punctate* by Landers (1955) and *E. weybridgensis* by Norton and catchpole (1976). There are several species of goats have also been reported including *E. ninakohlyakimovae, E. hirci, E. caprina, E. caprovina, E. alijevi, E. africiensis, E.*

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christenseni, E. punctatae, E. kocharli E. jolchijevi, E. apshronica, E. capralis, E. masseynsis, E. charlstoni, E. minasnsis and E. arloingi. *E. arloingi*and *E. ninakohlyakimovae*are considered as the highly prevalent pathogenic species (Silva and Lima 1998; Chartier and Paraud 2012). In Iraq Leiper (1957) first documented the *Eimeria spp.* in sheep, then Mirza (1970) recorded *E. ahsata, E. ninakohlyakimovan, E. intricate, E. faurei, E. carandailis, E. parva* and *E. granuolosa. E. ovinoidalis and E. Pallida* was first mentioned by Yakob et al. (1989).

Geographical Distribution and Prevalence

Eimeria has a worldwide distribution in sheep and goats, and it is difficult to define a specific geographical split between a single or numerous genus and species. As a result, sporadic occurrences of a single species with severe pathogenic consequences have been seen. Otherwise, some species have no pathogenic effect under normal conditions, and several publications have documented the occurrence of *Eimeria spp.* in sheep and goats around the world. Factors such as management, sanitary conditions, temperature, agroecology, climatic and environmental conditions, and the immunological response of the host, dosage of infection, and sampling duration can all affect the occurrence and distribution of Eimeriosis in different places (Khodakaram-Taftiand Hashemnia2017).

In Poland, 4.6-60% prevalence of *Eimeriaspp.* was recorded in sheep (Gorski et al. 2004), whereas in Austria the prevalence was 97-100% (Platzer et al. 2005), 43.1% (Reeg et al. 2005) and 37.61% (Hashemnia et al. 2014) and 74.8% prevalence was reported in Brazil (Berto et al. 2013). China, Zimbabwe, and Egypt recorded 91.5% in adult sheep and lambs, respectively (Kaya 2004; Yakhchali and Golami 2008; Mohamaden et al. 2018). In USA the prevalence of *Eimeriaspp.* in goat was 97% (Kahan and Greiner 2013), while in India it was 96.66% (Kaur et al. 2017), 65.07% in Egypt (Mohamaden et al. 2018), 55.99% in Pakistan (Rehman et al. 2011) and 73.91% in Brazil, respectively (Macedo et al. 2019).

In Iraq, distribution of *Eimeria spp.* varies according to the periods, regions and breed of sheep and goats. In Baghdad province the prevalence in sheep with Eimeriosis was 79.09% (Abd Al-Wahab,2003), while, in Diwaniya province it was reached to 1% in lambs as recorded by Dawood et al. (2008). On the other hand, Kalef and Fadl (2011) reported a prevalence rate of 49% in Baghdad province and Mohammed (2013) reported a prevalence rate of 67.5% in sheep in AlMuthana province. In Diyala province, the infection rate of 86.09% was recorded in sheep and 87.30% in goat (Mineet 2014), while Al-Sadoon(2018) recorded a prevalence rate of 84.16% in sheep in Wasit province. The rate of infection with *Eimeriaspp.* was affected by the way the farm was run and the number of cases of was found lower in large and closed farms. This did not necessarily mean that these farms had intensive systems, but it's likely because these farms used stricter hygiene measures and de-parasitization methods. Other factors, like differences in immunological competence due to differences in nutritional status, could have also played a role (Knox and Steel 1996). Furthermore, inadequate hygienic sanitation may be regarded as a risk factor for Eimeriosis, as it can increase the duration and amount of infection/exposure and the incidence of infection owing to contaminated food and water. Furthermore, stress may also promote immunosuppressant conditions. The presence of non-cemented floors, a closed housing system, and a large herd size, resulted in the greater contamination of overcrowded animals and feeding and watering troughs(Altaf and Hidayatua 2014). Furthermore, there may be statistically significant differences between a body condition score and *Eimeriaspp.* infection; for example, Khan et al. (2011) found a greater infection rate in sheep with low body ratings compared to those with superior body ratings. On the other hand, there are positive connections between conditions such as temperature and the severity of infection in semiarid and subhumid regions (Balicka-Ramisz 1999). This correlation might be related to the effect of temperature on *Eimeriaspp.* sporulation rates (Graat et al. 1994). This correlation explained that temperature effect on sporulation rates of the *Eimeria spp.* (Graat et al. 1994). The breed susceptibility differences also affect the *Eimeria spp.* infection. Indigenous goats in Zimbabwe were found to be resistant to Eimeriosis, while Angora and wild goats were found to be more likely to get clinical Eimeriosis than dairy breeds goats (Chhabra and Pandey 1991).

Pathogenicity

Many factors affecting on the Pathogenicity of *Eimeria* such as thedose of oocysts ingestion, host cells destruction, location of parasite in hosttissues, stage of infection, general condition and age of host, and degree of immunity which may be acquired or natural (Kaneko et al.2008; Moreet al. 2011). Gregory et al. (1983) looked at sheep that had been infected with *E. crandallis* and *E. bakuensis.* They found that these parasites can cause the host cell to go through mitosis and can sometimes divide at the same time as the host cell. During an *E. crandallis* infection, parasites can also divide continuously at the same time along with the epithelial cells of the host. Cox (2009) discovered that heavy *Eimeria spp.* infections result in schizonts found in mucosa and submucosa cells with high destruction and haemorrhage when compared to light infections that affect intestinal mucosa with local absorption. On the other hand, some *Eimeriaspp.* infections resulted in superficial development with villi atrophy, that might be due to a decrease in epithelial cell lifetime and the surface area accessible for absorption, resulting in a lower feed efficiency. Typically, infection with different species of *Eimeria* at same time was common in the field and cause a sever pathological effects (Blood and Radostitis 1989).

Catchpole et al. (1975) detected that mixed *Eimeriaspp.* infection in sheep resulted in prolonged patency and increased oocyst production with or without clinical signs. In general, *E. ovinoidalis* is regarded as one of the most virulent species in sheep (Gregory et al. 1989; Abakar 1996). In goats, *E. arloingi*and *E. ninakohlyakimovae* are the most common pathogenic species (Cavalcante et al. 2012). Stress and environmental variables are key predisposing factors in *Eimeria* pathogenesis, and a research has shown that these factors are linked to recurrent outbreaks of Eimeriosis (Gul 2007). Sometimes lambs and kids that treated with corticosteroids can convert subclinical infections to acute clinical infection (Gasmir 2005). On the other hand, schizonts growth cause damage in the caecum, which cause most numerous and mucosal polyps in sheep (Taylor and Catchpole 1994).

Clinical Signs

Different experimental studies showed different clinical signs in lambs and kids infected with Eimeriosis without prominentdifferences when used inoculated doses (Dai et al. 2006).The initial clinical symptom of Eimeriosis infection include the abrupt acute diarrhoea with bad odours and stools including mucus and blood, as along with an increasing loss of body weight (Blood and Radostitis 1989). According to a study,palemucous membranes, weakness, staggering, dyspnea, dehydration, andrecumbency were also reported in diseases animals(Mohamed et al.1990). While Abakar (1996) noted an appetite, dullness, pale mucous membranes, and minor pyrexia as clinical indications of acute Eimeriosis, leading to a disruption of the digestive system resulted in the release of water, electrolytes, and protein (Reid et al. 2012). Several lambs may eventually die on dehydration because of diarrhea and lose of appetite while, some lambs die with profuse watery diarrhea (Taylor et al.2007).

Diagnosis

Eimeriosis may be diagnosed in sheep and goats based on a case history, clinical indicators, gross lesions, necropsy results, and microscopic analysis of faeces by flotation method using various floatation liquids. So, a necropsyand recognized schizonts in lesions make a positive diagnosis (Levine1973). In the acute phase of Eimeriosis, the presence of a large number of sporozoites may lead to the tissue loss, resulting in the formation ofmerozoites that are failed to locate and invade new cells in order to grow before any oocysts form (Gregory et al.1983). Typically, *Eimeria* can easily be diagnosed through faecal examination using floatation technique (Levine 1961; Menezes and Lopes 1995).

Molecular Characterization of Eimeria spp.

The use of available tools in molecular biology is important to detect any parasitic infection that may infect human and animals and is important in modern Veterinary Diagnostic Parasitology comparing with the techniques used in past (Zarlenga and Higgins 2001). So, in the past, studies that looked for *Eimeriaspp*. used either traditional characteristics or a combination of traditional characteristics and other methods, such as the electrophoretic variation of enzymes in avian *Eimeriaspp.*, which uses variation in DNA sequences. The PCR-based assay has also been described, which could be used to identify *Eimeria spp.* (Viljoen and Nel 2002). The development of novel DNA-based diagnostic tests might expedite and simplify the identification of *Eimeriaspp.*, while the application of the PCR technique is changing the detection of pathogens (Erlich et al. 1991). According to Al-Sadoon (2018), the molecular study revealed the highest infection rate of *Eimeria spp.* of sheep at Wasit-province, Iraq via PCR on sheep faecal samples (84.16%), and phylogenetic tree analysis of the common four Eimeria species (*E. ovinoidalis, E. crandalis, E. ahsata, and E. weybridgensis*) has been disclosed employing multiplex PCR. The total infection rate of *Eimeria spp*. through PCR analysis showed a significant increase between species and included 57.42% positive samples, with *E. ahsata* having a higher infection rate (53.44%) followed by *E. ovinoidalis* (29.31%), *E. weybridgensis* (12.93%) and *E. crandallis* (4.31%), respectively.

Molecular characterization of *Eimeria spp.* by Shaheed (2021) in Basrah Province, Iraq

This study foundeleven *Eimeria spp.* in sheep and six *Eimeria* species in goats, respectively. This recognition depends on the shape and structure of isolated oocysts under microscope as: *E. ovinoidalis, E. crandallis, E. ahsata, E. weybridgensis, E. bakuensis (ovine), E. intricata, E. faurei, E. pallida, E. granulosa, E. parva* and *E. marsica*in sheep, while *E. arloingi, E. ninakohlakimovae, E. hirci, E. christenseni, E. aspheronica*and *E. capralis*in goats. Sporulation time of isolated oocysts was recorded by using Sugar solution in flotation, maturation, growth and diagnosis of *Eimeria* as a substitute method to potassium dichromate and formalin, that usually use in sporulation of *Eimeria spp.* The sugar is known as a nutritional substance with no caution or side effects compared to the potassium dichromate which is a carcinogenic substance while the formalin is also reported to be a harmful chemical to the human respiratory system. The results were astonished by using the sugar solution, as the rate of sporulation was estimated of 100% compared to the potassium dichromate which was observed giving a lower rate of only 30% of sporulation. In addition, the characteristic of *Eimeria* were very clear as a cyst that sporulated in the sugar solution compared to the cysts where sporulated in the potassium dichromate which was unclear under light microscope.The time of sporulation was continued from 1 day to 5 weeks with sugar solution, in comparison to 7 to 12 days with potassium dichromate. The result showed *E. bakuensis* and

E. parva of sheep and *E. arloingi, E. ninakohlakimovae, E. hirci, E. christenseni, E. capralis*of goats need three days or more to begin sporulation, while the other *Eimeria* species need less than three days to begin sporulation. According to the result of phylogenetic analysis there were nine *Eimeria spp*. recognized from twenty-five PCR positive fecal sample of sheep. *E. ovinoidalis, E. ahsata, E. crandallis, Eimeria spp. voucher* and *E. bovis* infected the cattle, *E.hirci* and *E. christenseni* infected the goats and *Eimeria labbeana*-like infected the birds and were recorded as a new species, and sheep infected with nonspecific species which was first record as a new species of *Eimeria* at Basrah province. It can be noticed that all isolates of *Eimeriaspp.* showed 92.54-99.51% similar identity with *Eimeriaspp.* isolated from different countries and recorded in GenBank, and it showed close association with the isolates detected from Iran and Jordon.

A- Evolutionary Relationships of *Eimeria spp.* Isolated in BasrahProvince, Iraq

The Neighbor-Joining method was applied to generate an estimate of the evolutionary history of the taxa that were investigated, and the bootstrap consensus tree that was derived from 500 different iterations of the analysis was selected in order to symbolize the evolutionary history of the species. When a bootstrap replicate is done, branches that belong to partitions that haven't been replicated in more than 50% of them are collapsed. Next to the branches are the percentages of duplicate trees in which related taxa were grouped together in the bootstrap test (500 times). The evolutionary distances were calculated using the Jukes-Cantor method. The research used 24 nucleotide sequences with codon locations 1^{st}+2^{nd}+3^{rd}+Noncoding in units of the number of base substitutions per site. All spots with blanks or missing information were taken out. In the end, there were a total of 268 locations in the dataset. MEGA7 was used to do an analysis of evolution. Fig. 1 shows phylogenetic analysis of *Eimeria spp.* isolated from small ruminants by using bootstrap consensus tree and Fig. 2 shows phylogenetic tree by using Neighbor-Joining method. Molecularly, all species found and recorded for the first time inBasrah province by using novel primers. Likewise, the normal host of *E. labbeana*are birds but it was isolated from sheep showing greater similarity with other strain submitted at GenBank from Iran, Jordon and Turkey. The results showed that these were neighboring countries and movement of animal in these countries by following import and export laws allowed the transmission of *Eimeria* and other parasitic infections.The evolutionary history of the studied taxa was figured out by using Neighbor-Joining method (Saitou and Nei 1987). The history of detected isolates was shown by the bootstrap consensus tree figured out from 500 replicates (Felsenstein 1985) and evolutionary distances were found using the Jukes-Cantor method (Jukes and Cantor 1969). Table 1 shows the percent identity of detected isolates with sequences available in GenBank.

B- *Eimeria* species detected in sheep

Eimeria ovinoidalis: Oocysts with an ellipsoidal form, smooth wall, colourless to pale-yellow, no polar cap, present inconspicuous micropyle, mean size 26.5±0.8× 20.3±0.8 having range 27.5 – 20 × 21.5 – 15 µm with sporulation period 1-3 days (Fig. 3).

***Eimeria crandallis*:** Oocysts are subspherical to broadly ellipsoidalshape and has smooth wall, with a micropyle, which may be distinct or indistinct and a micropylar cap, pale yellowish in color. Mean size 25.0±1.1×19.1±0.8 having range 27.5 – 18.5) × (20 – 12.5 µm and 1-3 days assporulation time (Fig. 3).

Eimeria weybridgensis: Oocysts are ellipsoidal to subspherical shape,a smooth wall, colorless or pale yellow. micropyle and polar cap present,mean size 31.0±1.5×20±0.7, with range 34.5 –24.5 × 24 – 20 µm, and 1-3 days as sporulation time (Fig. 4).

***Eimeria parva*:** Oocyst's shape is spherical to subspherical, smoothwall colorless to pale yellow, Polar cap absent, Micropyle absent, mean size 18.9±1.0× 15.6±1.0, with range 22-10 × 18-7.5 µm and 3-5 days as sporulation time (Fig. 4).

Eimeria ahsata: Oocysts are ellipsoidal shape, a smooth wallyellowish brown color, with distinct polar cap, and micropyle. mean size36.4±1.8× 24.1±1.3, with range 42.5-27.5×25–22.5 µm and 2-3 days as sporulation time (Fig. 5).

***Eimeria faurei*:** Oocyst is oval, pale-yellowish-brown in colour, coated with a smooth layer, no polar cap and prominent micropyle, mean size 32.1±0.6× 23.2±0.7, with range 37–22.5×27-20 µm and sporulation period 1-3 days (Fig. 5).

Eimeria bakuensis: Oocysts are ellipsoidal shape, pale yellowishbrown, micropyle and micropylar cap present, sporozoites lying head to tail in sporocyst, mean size 31.4±0.9× 18.9±0.6, with range 36 –20 × 24 – 15 µm, and 2-4 days as sporulation time (Fig. 6).

***Eimeria marsica*:** Oocysts are ellipsoidal shape, colorless slightlygreyish or pale yellow with smooth wall, with micropyle (indistinct) which may have an inconspicuous micropylar cap, mean size 22.7±0.4 × 15.7±0.7, with range 22.5-18.5×15-8 µm and 3 days as sporulation time (Fig. 6).

***Eimeria intricata*:** Oocyst are ellipsoidal shape or slightly ovoid, brownish yellow to dark brown in color, with thick wall that is granular and transversely striated, micropyle in the outer layer, a micropylar cap, mean size 48.0±2.3× 37.7±1.8, with range 56 – 40 ×41 – 30 µm, and 1-3 days as sporulation time. (Fig. 7).

Eimeria granulosa: Oocysts are urn-shaped, with a large micropleand micropylar cap at the broad end, yellowish-brown in color with twosmooth layers, mean size 33.6±1.4×22.1±1.4, with range 35 - 22 × 25 - 17.5 µm, and 1-2 days as sporulation time. (Fig. 7).

Table 1: Sequence identity with Accession number of strain in GenBank

	Student code	Sequence code	Identity	*Eimeria spp.*	Accession number At GenBank
1	G2	C2	92.45%	*Eimeria bovisHS02; HS18*	MZ562402.1
					MZ562419.1
2	G18	M2	93.30%	*Eimeria ahsata HS06; HS01*	MZ562403.1
					MZ562406.1
3	G6	M3	99.28%	*Eimeria crandallis HS03; HS10; HS16*	MZ562407.1
					MZ562412.1
					MZ562417.1
4	G16	M4	99.28%	*Eimeria crandallis HS07; HS08; HS09*	MZ562409.1
					MZ562410.1
					MZ562411.1
5	G5	M5	99.05%	*Eimeria crandallis HS21*	MZ562421.1
6	G19	M11	98.02%	*Eimeria christenseni HS17*	MZ562418.1
7	G20	M12	99.46%	*Eimeria hirci HS11*	MZ562413.1
8	G12	M13	99.01%	*Eimeria christenseni HS23*	MZ562405.1
9	C3	M14	97.98%	*Eimeria faure HS05*	MZ562408.1
10	C4	M15	98.47%	*Eimeria ovinoidalis HS12*	MZ562414.1
11	O6	M17	96.92%	*Eimeria sp.* RY-2016a HS04; HS13; HS14; HS20; HS22	MZ562400.1
					MZ562401.1
					MZ562415.1
					MZ562420.1
					MZ562422.1
12	S1	M19	93.80%	*Eimeria christenseni HS15*	MZ562416.1
13	S4	M20	99.28%	*Eimeria ovinoidalis HS19; HS24*	MZ562404.1
					MZ562423.1

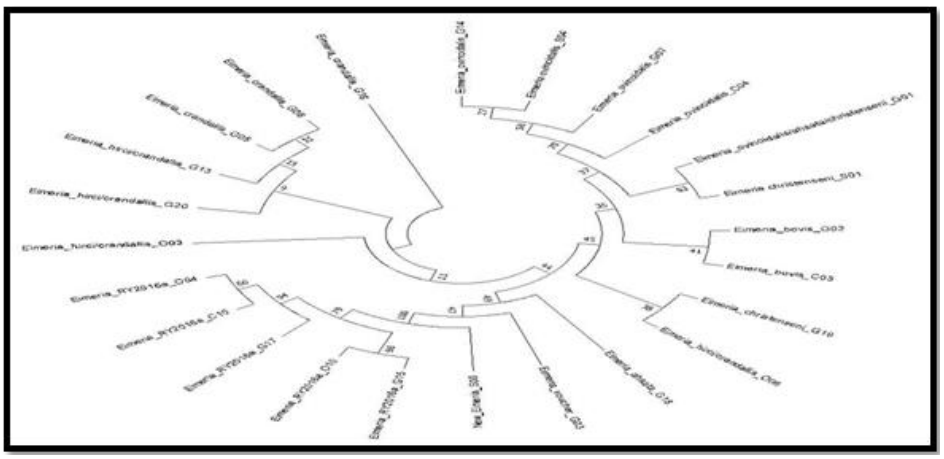

Fig. 1: Phylogenetic tree analysis (bootstrap consensus tree)

***Eimeria pallida*:** Oocysts are ellipsoidal, smooth wall colorless topale yellow or yellowish green, Polar cap absent, Micropyle absent, mean size 19.8±0.6×16.8±1.2, with range 20–12 × 15 - 8 µm, and 1-3 days as sporulation time (Fig. 8).

Eimeria Species Detected inGoats

***Eimeria ninakohlyakimovae*:** Oocysts are ellipsoidal or slightly subspherical, thin-walled, colorless, without micropyle or micropyle cap mean size 23.5±1.0×16.0±1.2, with range 24.3–20×19.5–14 µm and sporulation time is 1–4 days (Fig. 9).

Eimeria christenseni: The oocysts are ovoid or ellipsoidal, colorless topale yellow, with a micropyle and micropyle cap. mean size30.1±1.6×17.1±0.3, with range 44-27×31-17 µm and sporulation time is 3-6 days (Fig. 9).

Eimeria aspheronica: Oocysts are ovoid, greenish to yellow brown,with a micropyle but without a micropyle cap, mean size 24.6±0.3×17.5±1.2, with range 37-24 ×26-18 µm, and sporulation time is 1–2 days (Fig. 10).

Eimeria hirci: Oocysts are ellipsoidal to subspherical, light brown tobrownish yellow, with a micropyle and micropyle cap, mean size22.8±0.3×14.2±1.1, with range 23-18×19-14 µm, and sporulation time is 1–3 days (Fig. 10).

***Eimeria arloingi*:** Oocysts are ellipsoidal or slightly ovoid, with athick wall. a micropyle and micropyle cap present, mean size 29.2±1.6×17.1±1.1, with range 42-17×19-14 µm and sporulation time is 1–4 days (Fig. 11).

Eimeria capralis: Oocysts are ellipsoidal with a distinct micropylecap, but without micropyle having mean size 29.5±1.5×19.6±0.3**,** with range 34-25× 24.5-19.5 µmand 5 days as sporulation time (Fig. 11).

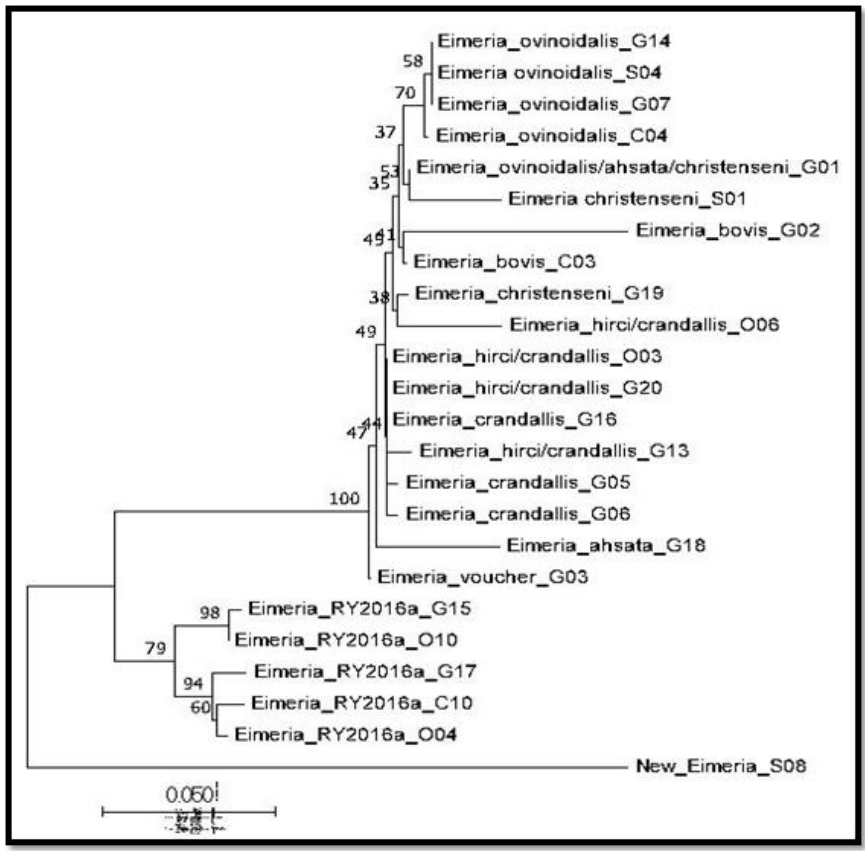

Fig. 2: Phylogenetic tree analysis (Neighbor-Joining method)

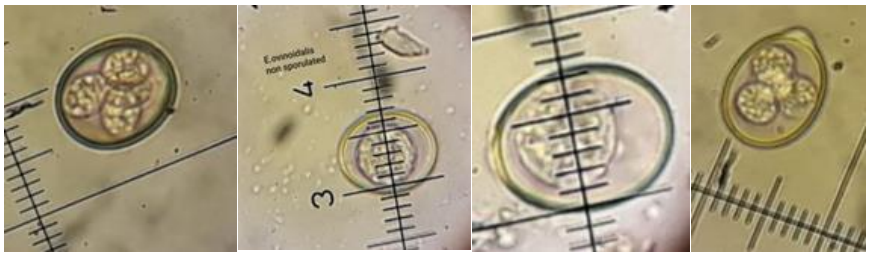

Fig. 3: Sporulated andnon sporulated Oocyst of *E. ovinoidalis* and*E. cran*

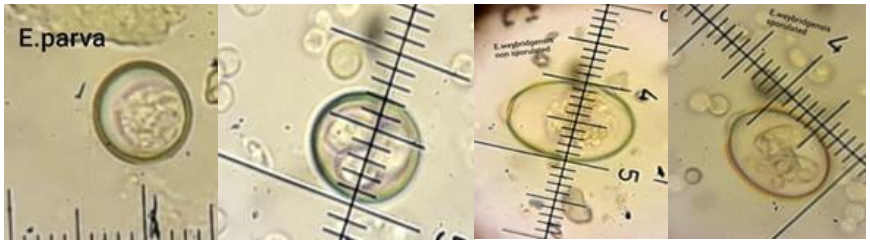

Fig. 4: Sporulated andnon sporulated Oocyst of *E. parva* and *E.weybridgensis* (40X)

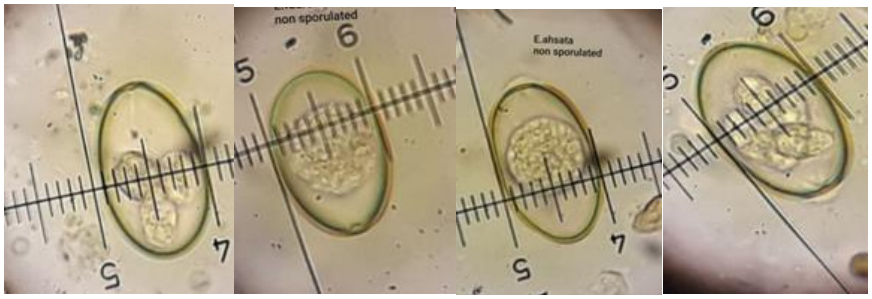

Fig. 5: Sporulated andnon sporulated Oocyst of *E. faurei* and *E. ahsata* (40X)

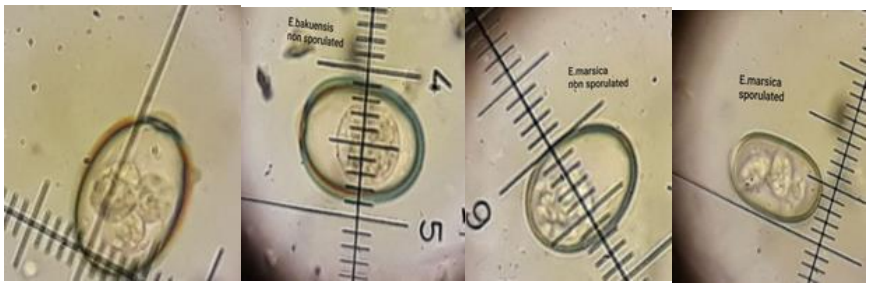

Fig. 6: Sporulated and non sporulated Oocyst of *E. bakuensis* and*E.marsica* (40X)

Fig. 7: Sporulated and non sporulated Oocyst of *E. granulosa and E. intricata* (40X).

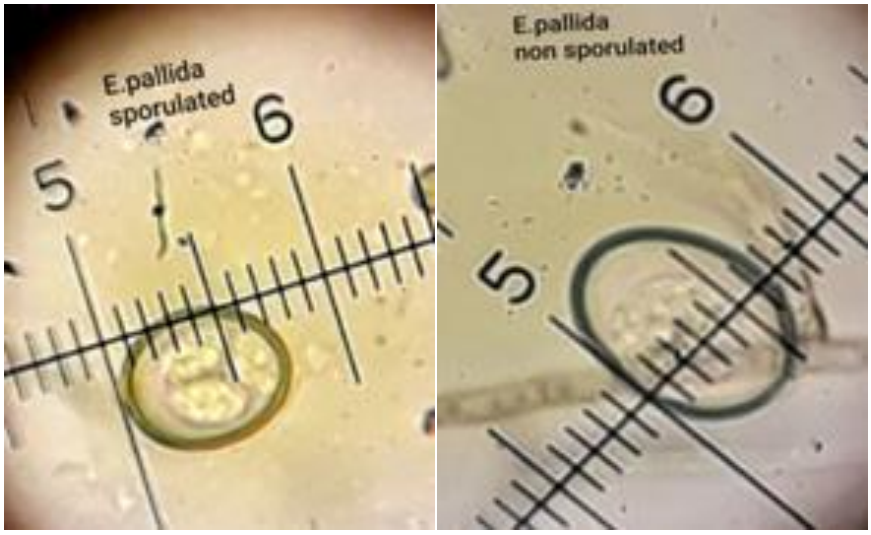

Fig. 8: Sporulated and non sporulated Oocyst of*E.pallida*(40X)

Fig. 9: Sporulated and non sporulated Oocyst of *E. christenseni and E. ninakohlyakimovae* (40X)

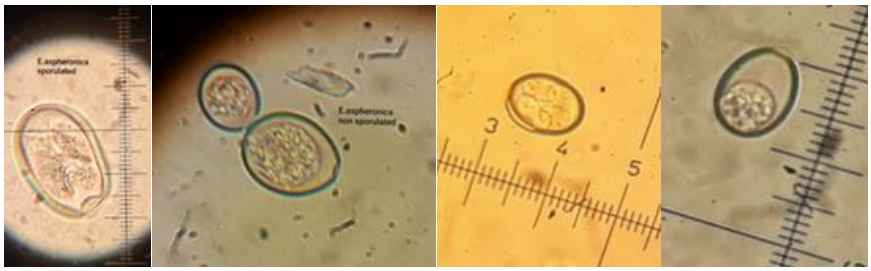

Fig. 10: Sporulated (100X) and non sporulated (40X) Oocyst of *E. aspheronica and E. hirci*(40X)

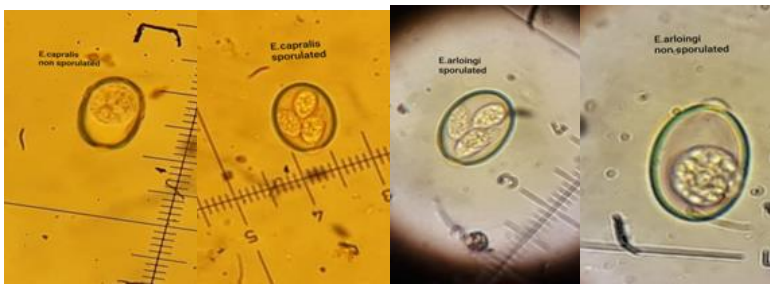

Fig. 11: Sporulated and non sporulated Oocyst of *E. capralis and E. arloingi*(40X)

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