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RESEARCH ARTICLE

Prevalence and Phylogenetic Analysis of *Eimeria* Species in Sheep and Goats in Sharkia Governorate, Egypt

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ABSTRACT

Eimeria spp. infections are one of the most economically significant diseases of sheep and goats. This study aimed to identify *Eimeria* spp. and its prevalence in sheep and goats reared under the intensive system in Sharkia Governorate, Egypt. Faecal samples (n=125) from sheep (n=64) and goats (n=61) were collected in three main rearing cities. Floatation technique was used to detect the coccidian oocysts in collected samples. Oocysts were allowed to sporulate and then identified based on morphological features and molecular analysis. Prevalence of *Eimeria* spp. infection was 60.9% in sheep (39/64) and 83.6% (51/61) in goats. Nineteen *Eimeria* spp. were detected in both sheep and goats. In sheep, ten species (*E. ahsata*, *E. pallida*, *E. intricata*, *E. ovinoidalis*, *E. marsica*, *E. bakuensis*, *E. faurei*, *E. granulosa*, *E. crandallis* and *E. parva*) were identified with *E. ahsata* being the most prevalent (26.6%). However, only nine species (*E. arloingi*, *E. alijevi*, *E. ninakohlyakimovae*, *E. hirci*, *E. christenseni*, *E. aspheronica*, *E. jolchijevi*, *E. caprina* and *E. caprovina*) were found in goats and *E. arloingi* was the most common species (45.5%). Mixed infections with *Eimeria* spp. were detected in 43.6% (17/39) and 74.5% (38/51) of examined sheep and goats, respectively. The sequence of internal transcribed spacer-1 (ITS-1) region of *E. ahsata* was 100% similar to ovine *E. ahsata*, and clustered in a single clade with *E. cardinalis* and *E. faurei*. However, *E. arloingi* was 100% similar to *E. arloingi* of goat and clustered with bovine *E. ellipsoidalis*. Our results showed that coccidial infection in sheep and goats reared under intensive production in Sharkia Governorate is high, suggesting that a strategy for controlling the disease is required in Egypt.

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INTRODUCTION

Coccidiosis is an economically important parasitic disease that causes high morbidity in both domestic and wild animals (Molnár *et al.,* 2012; Yan *et al.,* 2013). It is caused by *Eimeria* spp. which invades the small and large intestine of the hosts, causing anemia, electrolyte losses and diarrhea (Foreyt, 1990). Infection of small ruminants with *Eimeria* spp. is common; however, lambs and kids are the most severely affected (Kara, 1999). Coccidiosis in sheep and goats is asymptomatic; however, some *Eimeria* spp. showed clinical signs including diarrhea, poor weight gain, rough hair coat, weakness and decrease productivity (Radostits *et al.,* 1994; Silva *et al.,* 2014a). Furthermore, infected sheep and goat under stress conditions may die (Koudela and Boková, 1998), and mortality rates in lambs and kids can reach up to 10% (Soulsby, 1982). Animals recovered may continue to show poor production of milk, meat and fiber (Radostits *et al.,* 1994).

Many *Eimeria* spp. have been reported in sheep (~15 species) and goats (~17 species) worldwide (Khodakaram-Tafti *et al.,* 2013). However, the most prevalent and pathogenic *Eimeria* spp. in sheep and goats are *E. ahsata* and *E. arloingi*, respectively (Jalila *et al.,* 1998). Traditionally, identification of *Eimeria* spp. has been

performed by microscope using the morphological analysis of the sporulated oocysts including oocyst residue, size, sporulation time, polar cap and micropyle (Kawahara *et al.,* 2010; Nahavandi *et al.,* 2016). However, the morphological approach has some practical limitations related to the sensitivity and time (Carvalho *et al.,* 2011) and the skills of laboratory technician (Al-Habsi *et al.,* 2017). Therefore, molecular tools such as the sequencing of internal transcribed spacer-1 (ITS-1) and 18S rDNA regions have been introduced to overcome the limitations of the traditional methods (Kawahara *et al.,* 2010).

In Egypt, small ruminant production is one of the key sectors in the food supply chain. The overgrowth of the population and its demands for livestock products were the key driver of the development of small ruminant intensive production system in Egypt (McDermott *et al.,* 2010). Sheep and goats reared under intensive production system are at higher risk of *Eimeria* spp. infection (Cavalcante *et al.,* 2012). Some studies have shown that *Eimeria* spp. infection in sheep and goats under extensive production system is common (Mohamaden *et al.,* 2018; Hassan *et al.,* 2019). However, there is little information about sheep and goats coccidial infection under the intensive production system in Egypt. The current study aimed to identify *Eimeria* spp. and its prevalence in sheep and goats reared under intensive system in Sharkia Governorate, Egypt.

MATERIALS AND METHODS

Study region: This survey was performed in Sharkia Governorate in northern Egypt. Sharkia Governorate is the third most populous governorate in Egypt and located in the Nile Delta region at 30.7°N, 31.63°E, at 120 Km from the capital Cairo (Fig. 1). The mean temperature was 16°C and rainfalls ranged from 50 to 200 mm. This governorate has a strong agriculture industry and provides the foundation for livestock industry development in Egypt. It has a high density of sheep, goats, cattle and camels, which are mainly used for meat and milk production.

Study design and sampling: A cross-sectional study was conducted in Sharkia Governorate to collect faecal samples from sheep and goats farms in three main sheep and goats rearing cities (Zagazig, Bilbies and Minya El Qamh). The sample size was calculated to be 125 to estimate *Eimeria* species prevalence in the sheep and goat farms under intensive system in Sharkia province with 95% confidence and 5% precision, assuming a prevalence of 9% based on previous works on the same province. A total of 125 faecal samples were collected between December 2017 and November 2018. Briefly, 4 to 5 faecal samples were collected randomly from 4 to 6 representative sheep and goat's farms in each city. Faecal samples were obtained directly from rectum of sheep and goats using gloves or immediately after defecation. Samples were collected in plastic containers, marked with species, farm, animal ID and date of collection, then transported in a cooler for macro and microscopical analysis at the laboratory. Samples were collected from 64 sheep including 25 lamb and 61 goats including 27 kids. The research protocol was accepted by the Institutional Animal Care and Use Committee, Zagazig University, Egypt.

Recovery and identification of *Eimeria* oocysts: Faecal samples were tested with floatation technique for detection of coccidian oocysts using saturated saline (Soulsby, 1982). After examination, positive samples were filtered through sieves covered with folded gauze and moved into 2.5% (w/v) aqueous potassium dichromate solution to allow sporulation at room temperature for 7 days. Morphological characteristics (shape, size, colour and existence or lack of micropyle and its cap) of the oocysts and sporocysts have been used to describe the coccidian oocysts species (Soulsby, 1982; Taylor *et al.,* 2007).

DNA extraction: Commercial kit (QIAamp DNA stool Mini Kit, QIAGEN, Hilden, Germany) was used for DNA isolation from oocyst positive specimens. Briefly, 1.4 ml of buffer (ASL) was applied to the faecal sample (220 mg) and left for 5 min at 70°C, homogenized for 6 min using tissue lyser and then centrifuged for 1 min at 14,000 rpm. A one Inhibit Ex tablet was added to the collected supernatant, vortexed and then centrifugation (14,000 rpm /3 min) after incubation for 1 min at room temperature. Collected supernatant (200 µl), proteinase K (15 µl) and lysis buffer (200 µl) were incubated for 10 min at 70°C. After incubation, 200 µl of absolute ethyl alcohol was poured and the mixture was run through filter column at 14,000 rpm for 1 min and washed following manufacturer's recommendations. Elution buffer (100 µl) in the kit was used for elution of extracted DNA and preserved at -20°C for later analysis.

Amplification of ITS-1 rDNA region: The primers F (5′-GCAAAAGTCGTAACACGGTTTCC-3′) and R (5′-CTGCAATTCACAATGCGTATCG-3′) were used for amplification of the ITS-1 region of the extracted DNA by conventional PCR (Khodakaram-Tafti *et al.,* 2013). The reactions took place in a thermal cycler (Biometra T3 Thermocycler, Göttingen, Germany) with an initial denaturation for 5 min at 94°C. An amplification step was performed for 35 cycles of denaturation at 94°C for 30 seconds, and annealing at 56°C for 1 min, then an extension for 1 min at 72°C, with a further extension step at 72°C for 10 min (Verma *et al.,* 2017). The PCR products from ITS-1 rDNA were separated on 1.5% agarose gel in 1x TBE buffer at 37°C with 5V/cm of gradients. Fragment size was detected using DNA Ladder of 100pb (Qiagen, Germany). After that, a gel documentation system (Alpha Innotech, Biometra) was used to photograph this gel.

Sequencing of the ITS-1 rDNA and phylogenetic analysis: The ITS-1 DNA of *E. ahsata* and *E. arloingi* recovered from sheep and goats, respectively, were sequenced. The extraction kit (QIA quick PCR product kit, Qiagen Inc. Valencia CA) was used to purify the size of these bands following manufacturer's instructions. The sequencing kit (Bigdye Terminator V3.1 cycle kit, Perkin-Elmer, Foster City, CA, USA) was used for sequencing process with the help of 3130 Applied genetic analyzer Biosystems (HITACHI, Tokyo, Japan). The sequences obtained from the ITS-1 region were compared with the existing *Eimeria* spp. ITS-1 region sequences on GenBank. Sequences were placed in GenBank with accession numbers MN249723 and MN249722. The nucleotide sequences were aligned with other ITS-1

region sequences available at GenBank by the ClustalW method. The neighbour-joining approach was used to build the phylogenetic tree through MEGA software (version 6) (Tamura *et al.,* 2013) using one thousand bootstrap values.

Statistical analysis: The prevalence of coccidia infections was determined in sheep and goats from the oocyst positive ratio to the total number of sheep and goats examined. The variations in coccidiosis prevalence between sheep and goats, as well as between age groups within each species, were analyzed using the chi-square (χ^2) test. STATA ver. 16 for Windows (Stata Corp., College Station, TX) were used to perform statistical analysis with a p-value <0.05 as statistically significant.

RESULTS

Of the 64 sheep and 61 goats involved in the study, a total of 125 faecal samples were collected. There were 35.2% (44/125) males, 64.8% (81/125) females, including 41.6% (52/125) lamb/kids and 58.4% (73/125) adult sheep/goats. Most sheep and goats (109/125) involved in the present study were apparently healthy and only 12.8% (16/125) of the animals (nine sheep and seven goats) showed a varying degree of dehydration, emaciation and diarrhea. The prevalence of *Eimeria* spp. infection in sheep was 60.9% and 83.6% in goats (Table 1). However, there was a statistically significant (P=0.005) difference in prevalence among sheep and goats. The prevalence of *Eimeria* spp. infection in lambs (76.0%) was significantly (P=0.048) higher than those in adult sheep (51.3%). However, the prevalence of *Eimeria* spp. infection among kids and adult goats was not significantly different (P=0.767). Coccidian oocysts were detected in 52.2% (12/23) of male sheep faecal samples, and 65.9% (27/41) of female sheep faecal samples, and the difference was not statistically significant (P=0.282). In goats, the coccidian oocysts prevalence was 76.2% (16/21) in males and 87.5% (35/40) in females, and also no significant difference was observed (P=0.257). Infections with mixed *Eimeria* spp. were detected in 43.6% (17/39) and 74.5% (38/51) of examined sheep and goats, respectively (Table 1).

Totally, 19 *Eimeria* spp. were identified in examined sheep (10 species; Fig. 2) and goats (nine species; Fig. 3). Table 2 shows the frequencies of detection of each *Eimeria* spp. in sheep and goats. The most frequently detected *Eimeria* spp. in sheep and goats were *E. ahsata* (26.6%) and *E. arloingi* (47.5%), respectively.

The PCR products obtained from the ITS-1 region of the most prevalent *Eimeria* spp. in sheep (*E. ahsata*) and goats (*E. arloingi*) were successfully amplified. The length of the obtained DNA fragments was 444 bp and 439 bp for *E. ahsata* and *E. arloingi*, respectively. PCR products were sequenced and uploaded to GenBank under accession numbers MN249723 and MN249722 for *E. ahsata* and *E. arloingi*, respectively. The phylogenetic trees were created by the neighbour-joining approach (Fig. 4). Phylogenetic analysis of the ITS-1 sequences clustered *E. ahsata* in ovine clade and *E. arloingi* in caprine clade. Furthermore, *E. ahsata* and *E. arloingi* from this study shared 100% genetic similarity with *E. ahsata* (MG774401) and *E. arloingi* (MF356556) isolated in Mexico.

Table 1: Prevalence of *Eimeria* spp. infection in sheep and goats in Sharkia Governorate, Egypt

Animals	No. of examined	No. of infected animals (%)	No. of single infection (%)	No. of mixed infection (%)
Adult sheep	39	20 (51.3)	9 (45.0)	11 (55.0)
Lambs	25	19 (76.0)	13 (68.4)	6 (31.6)
Total	64	39 (60.9)	22 (56.4)	17 (43.6)
Adult goats	34	28 (82.4)	10 (35.7)	18 (64.3)
Kids	27	23 (85.2)	3 (13.0)	20 (87.0)
Total	61	51 (83.6)	13 25.5)	38 (74.5)

Table 2: Frequency of detection of different *Eimeria* spp. in sheep and goats in Sharkia Governorate, Egypt

Eimeria spp.	No. of positive	Prevalence (%)
Sheep (n = 64)		
E. pallida	10	15.6
E. marsica	8	12.5
E. ovinoidalis	9	14.1
E. parva	3	4.7
E. crandallis	4	6.3
E. faurei	5	7.8
E. granulosa	11	17.2
E. bakuensis	8	12.5
E. ahsata	17	26.6
E. intricata	10	15.6
Goats (n = 61)		
E. alijevi	21	34.4
E. ninakohlyakimovae	19	31.1
E. hirci	19	31.1
E. arloingi	29	47.5
E. jolchijevi	13	21.3
E. christenseni	19	31.1
E. aspheronica	15	24.6
E. caprina	12	19.7
E. caprovina	10	16.4

DISCUSSION

Eimeria spp. infection has been reported worldwide in sheep and goats (Chartier and Paraud, 2012). In the current study, the prevalence of *Eimeria* spp. infection in sheep was 60.9% and 83.6% in goats. The reported prevalence in sheep was nearly similar to the 57.7% reported recently in Egypt (Mohamaden *et al.*, 2018), the 57.5% in Iraq (Al-Saadoon and Al-Rubaie, 2018) and the 68.3% in Brazil (Souza *et al.*, 2015), but higher than the 19.2% and 43% reported in Iran and Pakistan, respectively (Khan *et al.*, 2011). However, the prevalence of *Eimeria* spp. infection in goats was lower than the 91.5% reported previously in Egypt (El-Manyawe, 1999), the 89.9% in Iran (Kheirandish *et al.*, 2014) and 98.1% in Southern Portugal (Silva *et al.*, 2014b), but a higher rate of infection has been reported in Egypt (60%; Mohamaden *et al.*, 2018) and India (23%; Das *et al.*, 2017). The variations in the prevalence of *Eimeria* spp. infection in sheep and goats might be due to the differences in management and hygienic conditions, temperature, agroecology, climate, immune state of the host, sample size, sampling period, breed susceptibility and techniques used to detect *Eimeria* spp. in different areas (Khodakaram-Tafti and Hashemnia, 2017).

In this study, the prevalence of coccidian oocysts was lower in adult sheep and goats compared to that in lambs and kids, which is consistent with previous observations (El-Shahawy, 2016). 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 sheep and goats compared with those of lambs and kids (Wang *et al.*,

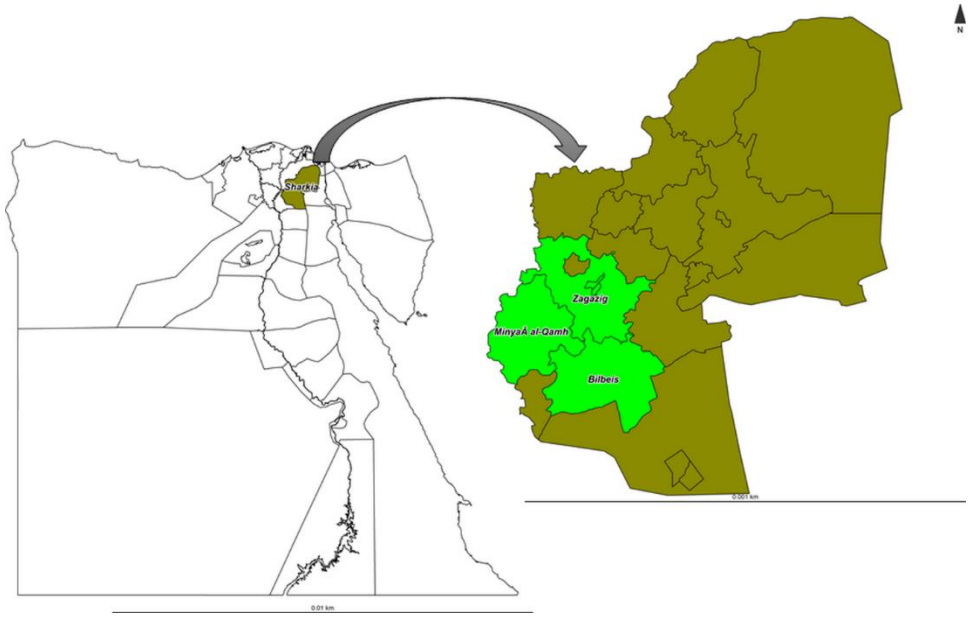

Fig. 1: Sharkia Governorate location in Egypt and the location of the three cities (light green colour) involved in the study.

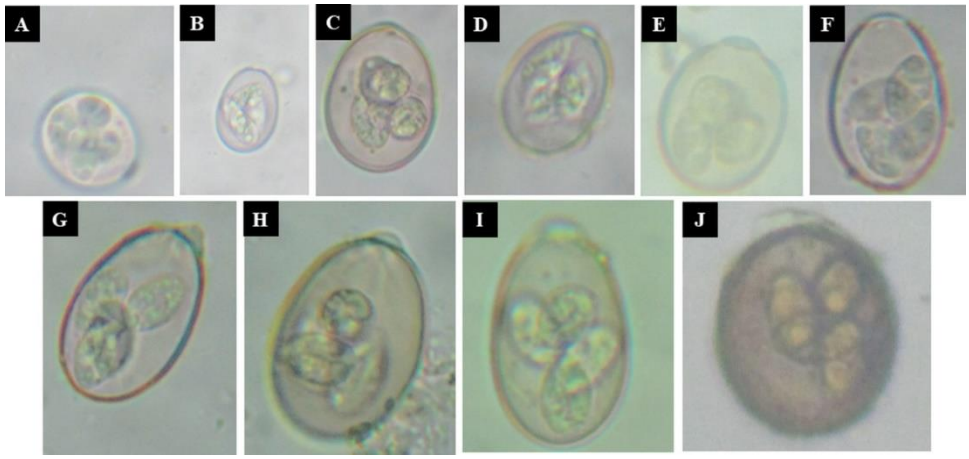

Fig. 2: Sporulated oocysts of *Eimeria* spp. (n=10) detected in sheep in this study. (A) *E. pallida*; (B) *E. marsica*; (C) *E. ovinoidalis*; (D) *E. parva*; (E) *E. crandallis*; (F) *E. faurei*; (G) *E. granulosa*; (H) *E. bakuensis*; (I) *E. ahasta*; (J) *E. intricata* (x400).

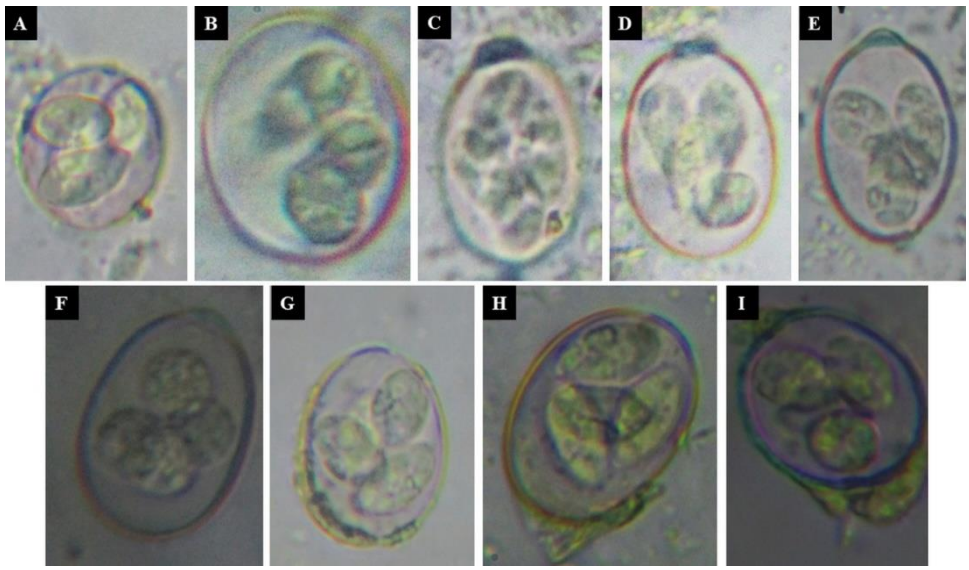

Fig. 3: Sporulated oocysts of *Eimeria* spp. (n=9) detected in goats in this study. (A) *E. alijevi*; (B) *E. ninakohlyakimovae*; (C) *E. hirci*; (D) *E. arloingi*; (E) *E. jolchijevi*; (F) *E. christenseni*; (G) *E. aspheronica*; (H) *E. caprina*; (I) *E. caprovina* (x400).

2010). Also, in this study, the rate of *Eimeria* spp. infection in female sheep and goats was higher than those in males. This finding is in agreement with previous studies (Rehman *et al.*, 2011; Mohamaden *et al.*, 2018), which reported that ewes and does are exposed to physiological stress in relation to pregnancy, giving birth and lactation that make it more susceptible to *Eimeria* spp. infection than males. The observed difference in the rate of mixed infections in sheep and goats was consistent with previous research reports (Kahan and Greiner, 2013; 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).

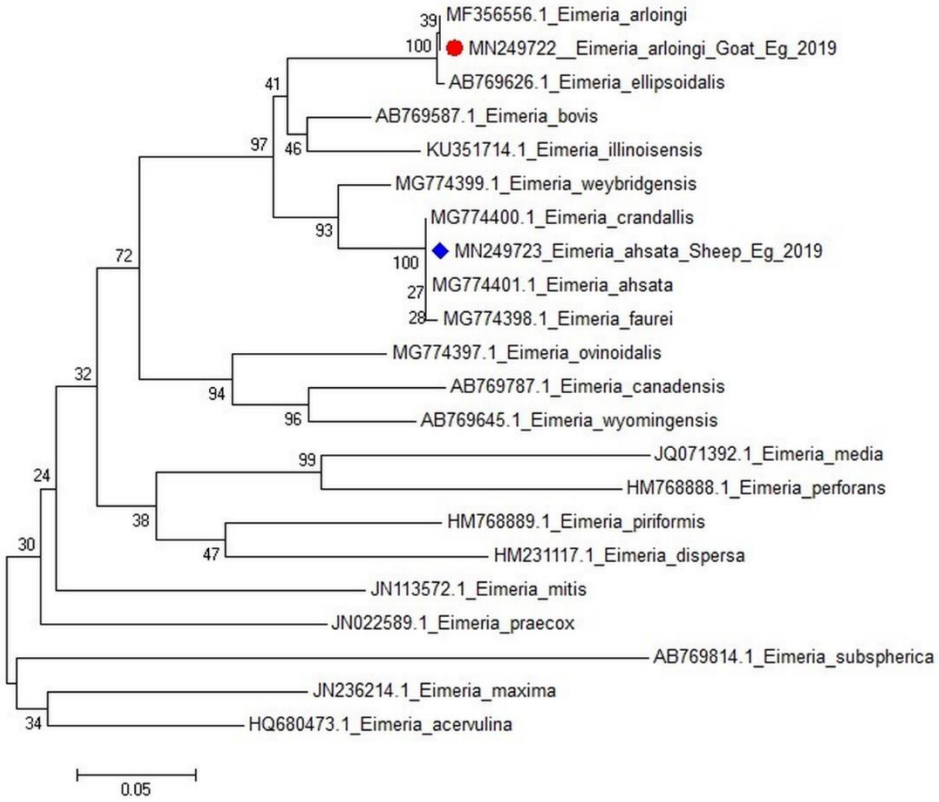

Fig. 4: Phylogenetic tree of the ITS-1 region sequences of *E. arloingi* (red circle) and *E. ahasta* (blue diamond) recovered from goat and sheep, respectively. Phylogenetic tree was generated using the Neighbour-Joining method and 1000 bootstrap values.

To date, nine and seven *Eimeria* spp. have been reported in sheep and goats in Egypt, respectively (Mohamaden *et al.*, 2018). But in this study, we found ten and nine *Eimeria* spp. in addition to the species previously reported in Egypt; *E. marsica* was detected in sheep and *E. caprovina* and *E. alijevi* were detected in goats. Although, pathogenic *Eimeria* spp. (*E.* ovinoidalis, *E. ahsata*, *E. crandallis* and *E. bakuensis*) and (*E. ninakohliyakimova*, *E. arloingi*, *E. christenseni* and *E. caprina*) were found in sheep and goats of current study, respectively, but most of the infected animals were apparently healthy. Furthermore, *E. ahsata* and *E. arloingi* were the most frequently found *Eimeria* spp. in sheep and goat, respectively, which is in line with other researcher's findings (Wang *et al.*, 2010; Al-Saadoon and Al-Rubaie, 2018; Mohamaden *et al.*, 2018). The phylogenetic analysis of the ITS-1 region reveled that *E. ahsata* found in sheep in this study was identical to ovine homologues *E. ahsata* (MG774401) from sheep reported in Mexico; while they were clustered together in a single clade with *E. cardinalis* (MG774400) and *E. faurei* (MG774398). On the other hand, *E. arloingi* detected in goats in the current study was also similar to caprine homologous *E. arloingi* (MF356556) from goats reported in Mexico and clustered in a single clade with *E. ellipsoidalis* of cattle (AB769626). These findings were consistent with other studies (Khodakaram-Tafti *et al.*, 2013; Trejo-Huitrón *et al.*, 2020), that reported the usefulness of ITS-1 region in differentiation between ovine and caprine *Eimeria* spp.

Conclusions: The present study provides basic data about the most prevalent *Eimeria* spp. among sheep and goats reared under intensive production system in Sharkia Governorate, Egypt. In addition, the present study reported significantly high rates of coccidial infection in sheep and goats in Sharkia Governorate, suggesting that a strategy for controlling the disease is required in Egypt.

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Authors contribution: EH, RA, WE contributed in the design and conception of the study. EH, RA, WE collected samples and performed the parasitological examination. IE carried out the statistical analysis. EH, RA, WE, IE wrote and revised the manuscript, and approved the final version.

REFERENCES

Al-Habsi K, Yang R, Ryan U, *et al.,* 2017. Morphological and molecular characterization of three *Eimeria* species from captured rangeland goats in Western Australia. Vet Parasitol Reg Stud Reports 9:75-83.

Al-Saadoon ZM, Al-Rubaie H, 2018. Traditional and molecular study for prevalence of coccidiosis in sheep in wasit-iraq Indian. J Natural Sci 8:14394-401.

Carvalho FS, Wenceslau AA, Teixeira M, *et al.,* 2011. Diagnosis of *Eimeria* species using traditional and molecular methods in field studies. Vet Parasitol 176:95-100.

Cavalcante ACR, Teixeira M, Monteiro JP, *et al.,* 2012. *Eimeria* species in dairy goats in Brazil. Vet Parasitol 183:356-8.

Chartier C, Paraud C, 2012. Coccidiosis due to *Eimeria* in sheep and goats, a review. Small Rumin Res 103:84-92.

Das M, Laha R, Goswami A, *et al.,* 2017. Gastrointestinal parasitism of goats in hilly region of Meghalaya, India. Vet World 10:81.

El-Manyawe S, 1999. Intestinal protozoal infection among goats in Egypt. Vet Med J 47:379-88.

El-Shahawy I, 2016. Two new species of coccidia, *Eimeria pavota* and *Eimeria egyptica* parasitic in white peacocks, Pavo cristatus (Galliformes: Phasianidae) in Egypt. Trop Biomed 33:437-45.

Foreyt WJ, 1990. Coccidiosis and cryptosporidiosis in sheep and goats. Vet Clin of N Am Food Anim Prac 6:655-70.

Hassan NM, Farag TK, El Ezz NMA, *et al.,* 2019. Prevalence assessment of gastrointestinal parasitic infections among goats in Giza Governorate, Egypt. Bull of Nat Res Cent 43:1-7.

Jalila A, Dorny P, Sani R, *et al.,* 1998. Coccidial infections of goats in Selangor, peninsular Malaysia. Vet Parasitol 74:165-72.

Kahan TB, Greiner EC, 2013. Coccidiosis of goats in Florida, USA.

Kara M, 1999. The prevalence of coccidian species in sheep in Kars province of Turkey. Trop Anim Health and Prod 31:161-5.

Kawahara F, Zhang G, Mingala CN, *et al.,* 2010. Genetic analysis and development of species-specific PCR assays based on ITS-1 region of rRNA in bovine *Eimeria* parasites. Vet Parasitol 174:49-57

Khan M, Rehman T, Iqbal Z, *et al.,* 2011. Prevalence and associated risk factors of *Eimeria* in sheep of Punjab, Pakistan. World Acad Sci Eng Technol 5:334-8.

Kheirandish R, Nourollahi-Fard SR, Yadegari Z, 2014. Prevalence and pathology of coccidiosis in goats in Southeastern Iran. J Parasitic Dis 38:27-31

Khodakaram-Tafti A, Hashemnia M, 2017. An overview of intestinal coccidiosis in sheep and goats. Rev Méd Vét 168:1-3.

Khodakaram-Tafti A, Hashemnia M, Razavi S, *et al.,* 2013. Genetic characterization and phylogenetic analysis of *Eimeria arloingi* in Iranian native kids. Parasitol Res 112:3187-92.

Koudela B, Boková A, 1998. Coccidiosis in goats in the Czech Republic. Vet Parasitol 76:261-7.

McDermott JJ, Staal SJ, Freeman H, *et al.,* 2010. Sustaining intensification of smallholder livestock systems in the tropics. Livest Sci 130:95-109.

Mohamaden WI, Sallam NH, Abouelhassan EM, 2018. Prevalence of *Eimeria* species among sheep and goats in Suez Governorate, Egypt. Int J Vet Sci Med 6:65-72.

Molnár K, Ostoros G, Dunams-Morel D, *et al.,* 2012. *Eimeria* that infect fish are diverse and are related to, but distinct from, those that infect terrestrial vertebrates. Infect Genet Evol 12:1810-5.

Nahavandi KH, Mahvi AH, Mohebali M, *et al.,* 2016. Molecular typing of *Eimeria ahsata* and *E. crandallis* isolated from slaughterhouse wastewater. Jundishapur J Microbiol 9: e34140.

Radostits O, Blood D, Gay C, 1994. Acute carbohydrate engorgement of ruminants (rumen overload) Veterinary Medicine A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 8th ed Baillière Tindall, London pp:262-9.

Rehman TU, Khan MN, Sajid MS, *et al.,* 2011. Epidemiology of *Eimeria* and associated risk factors in cattle of district Toba Tek Singh, Pakistan. Parasitol Res 108:1171-7.

Silva LM, Caro TM, Gerstberger R, *et al.,* 2014a. The apicomplexan parasite *Eimeria arloingi* induces caprine neutrophil extracellular traps. Parasitol Res 113:2797-807.

Silva LMRd, Vila-Viçosa MJM, Nunes T, *et al.,* 2014b. *Eimeria* infections in goats in Southern Portugal. Rev Bras Parasitol Vet 23:280-6.

Soulsby E, 1982. Helminths Arthropods and Protozoa of domesticated animals, 7th ed, Bailliere and Tyndall, London, UK pp: 765-6.

Souza LEBd, Cruz JFd, Teixeira Neto MR, *et al.,* 2015. Epidemiology of *Eimeria* infections in sheep raised extensively in a semiarid region of Brazil. Rev Bras Parasitol Vet 24:410-5.

Tamura K, Stecher G, Peterson D, *et al.,* 2013. MEGA6: molecular evolutionary genetics analysis version 6.0 Mol Biol Evol 30:2725-9.

Taylor M, Coop R, Wall R, 2007. Parasites of the respiratory system. In Veterinary Parasitology 3rd ed, Blackwell Publishing Ltd, Oxford, UK pp:195-9.

Trejo-Huitrón G, Bautista-Gómez LG, Martínez-Castañeda JS, *et al.,* 2020. Morphological characterization and first molecular identification of the eleven *Eimeria* species that infect sheep from Mexico. Parasitol Res 119:115-22.

Verma R, Sharma DK, Gururaj K, 2017. Molecular epidemiology and point mutations in ITS1 and 18S rDNA genes of *Eimeria ninakohlyakimovae* and *E. christenseni* isolated from Indian goats. Vet Parasitol Reg Stud Reports 9:51-62.

Wang C, Xiao J, Chen A, *et al.,* 2010. Prevalence of coccidial infection in sheep and goats in Northeastern China. Vet Parasitol 174:213-7.

Yan W, Wang W, Wang T, *et al.,* 2013. Simultaneous identification of three highly pathogenic *Eimeria* species in rabbits using a multiplex PCR diagnostic assay based on ITS1-5.8 S rRNA-ITS2 fragments. Vet Parasitol 193:284-8.